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U.S.S.R. LITERATURE ON AIR POLLUTION AND RELATED OCCUPATIONAL DISEASES, VOLUME 17. THE BIOLOGICAL EFFECTS AND HYGIENIC IMPORTANCE OF ATMOSPHERIC POLLUTANTS, BOOK 10

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Washington, D. C.

1968

PB 180522 T

U.S.S.R. LITERATURE ON AIR POLLUTION AND RELATED OCCUPATIONAL DISEASES

Volume 17

A SURVEY

by B. S. Levine, Ph. D.

THE BIOLOGICAL EFFECTS AND HYGIENIC IMPORTANCE OF ATMOSPHERIC POLLUTANTS

BOOK 10

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Springfield, Virginia

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The present English edition is a part of a survey conducted by
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supported by PHS Research Grant AP - 00176,
awarded by the Division of Air Pollution
of the U. S. P. H. Service

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PREFACE

The papers comprised in The Biological Effects and Hygienic Importance of Atmospheric Contaminants contain new data pertaining to the role "expanding popular chemistry" played in the industrial history of the USSR. Few data have been found in the literature on the harm that the high-tonnage-chemical-process industry can cause to the population. Whatever the branch of industry, little is known of the toxicity of its emissions--especially in low concentrations inhaled over a long time, or the methods of analyzing atmospheric air. Consequently, it has not been determined in what concentrations the new industrial emissions may be discharged into the atmosphere without adversely affecting the population's health.

It is understandable that under such circumstances the planning organizations send in numerous inquiries to the scientific research institutes engaged in problems of industrial environmental hygiene. Answers to requests for such information presuppose availability of appropriate experimental data on such questions as: what is the danger of a given type of industry for human health, in what combination and concentrations are harmful compounds discharged into the atmosphere, what are the maximum tolerable concentrations of such substances in the air, by what methods can these contaminants be determined, what measures can be recommended to reduce atmospheric pollutant concentrations, what sanitary zones can be considered adequate for industry of a given type, etc.

Some of these questions can be answered by studies conducted in some institutes, laboratories, and departments directed by the Project Commission of the Science Section of the USSR Ministry of Public Health. Papers presented in this collection are directly related to problems arising from the above indicated need for new and up-to-date environmental sanitary-hygienic information.

A. Ye. Kulakov investigated hexamethylenediamine, used in the production of nylon.

G. Kh. Ripp presents results of his interest in air pollution caused by divinyl, used in manufacturing synthetic divinyl-alpha-methyl-styrene rubber.

T. Yuldashev for the first time discusses the effects of ethylene oxide.

R. Ubaidullayev studied the hydrolytic phase of industry, dealing with cotton by-products, a raw material important to the Uzbekistan industry.

L. P. Slavgorodskiy has studied pollution by phthalic anhydride, and its toxicity.

I. S. Gusev made a comparative evaluation of such important compounds as benzene, toluene, and xylene, which are widely used as organic solvents and as raw material in synthesizing aromatic compounds.

M. I. Gusev and A. A. Minayev report on alpha-methylstyrene in the air in the proximity of synthetic rubber plants.

U. G. Pogosyan, Yu. Ye. Korneyev, and N. Z. Tkach investigated the production of acetone and phenol by the cumene method, a most interesting product of chemical synthesis with diverse discharges under the prevailing plant conditions.

M. S. Sadilova presents new and interesting studies on the effects of hydrogen fluoride on animals when administered chronically in microconcentrations.

The collection also contains papers on the chronic effects of acrylaldehyde, ammonia, and vanadium pentoxide.

BASIC PROBLEMS OF SANITARY ATMOSPHERIC AIR PROTECTION

Prof. V. A. Ryasanov

History indicates that air pollution as a sanitary hygienic problem was recognized about 100 years ago. The state of the atmosphere has since changed greatly, and the sources and types of pollution have become complex, and the problems to be solved are entirely different and immeasurably more complicated. This writer is in disagreement with the viewpoint of our foreign colleagues, who pessimistically view the air pollution problem and regard the increasing levels of urban pollution as unavoidable, and believe that mankind must become inured to this "civilization disease". Years of observation and reflection lead to a different conclusion. However, the striking transformations which progressively occur in this complex problem must be taken into consideration if a correct assessment of its tendencies is to be arrived at.

During the 19th and the first half of the 20th century mineral fuels were regarded as the principal source of air pollution due to the use in fireplaces, stoves, boilers, and electric stations, along with sulfuric acid, soot, and ash because these were the most dispersed, the most universal, and also the most harmful. By the middle of the present century the situation had begun to change. Many countries, especially the USSR, have taken radical measures to liquidate smoke-contaminated air: fireplaces and small boilers were replaced by heat supplied from central power stations, efficient coal-burning processes were developed which minimized the products of incomplete combustion, ash catchers of high purification degree were installed, the sulfur content of fuel was lowered, and, finally, mineral fuel was replaced by natural gas, both in large power stations and in homes; industrial and domestic processes were electrified by replacing smoking fuel with electric power. By the middle of the 20th century the problems of "smoke" in the real sense of the word was theoretically solved, and in many cities of the economically developed countries it was solved in practice. Newly developing nations should solve their "smoke" problem by introducing district heat, gas, and electric facilities or centers. There are also other auxiliary means.

However, in addition to the process of fuel combustion there are other factors that play a known, though limited, role in air pollution, such as the metallurgical industry's high-capacity hearths; however, such pollution sources, located in particular geographic areas, have a limited effect since the pollutants did not extend beyond a certain environmental zone. The nonferrous metallurgy has also created dangerous pollutant foci. As the oxide deposits became exhausted toward the end of the 19th century, the metallurgic industry was compelled to exploit low grade polysulfate containing ores, which led to severe sulfurous air pollution, which broke up the ground protecting surface layer for miles around the nonferrous ore sources. Polysulfide ores yield a dust of highly complex composition, some components of which are highly toxic to man and animals. The pyrometallurgical process transformed these extraneous metallic elements into smoke, thereby increasing their dispersion and enhancing the dust danger, while oxidation of these elements further increased their toxicity. With the advent of light metals such as aluminum, magnesium, beryllium, etc., their harmful compounds became known, such as fluorine compounds connected with the phenomenon of fluorosis among children, and resins containing large amounts of 3, 4-benzopyrene, and beryllium which caused in the nearby populated areas a pathologic condiction known as berylliosis. However, the above mentioned serious antihygienic conditions came into being as the result of the metallurgical industry development and were looked upon as a local phenomena as long as fuel combustion was a national problem. It is, therefore, understandable why smoke should compel the attention of all authorities concerned.

Metallurgical enterprises as sources of air pollution and the sanitary hygienic problems they created are of equal importance theoretically and practically. Theoretically this problem can be solved by the judicious use of raw material. Instead of being discharged into the atmosphere as vast amounts of SO_2 generated in smelting, these concentrated gases should be utilized as a raw material by plants producing sulfuric acid and plants related to the nonferrous metallurgy. Instead of being used whole, polymetallic ore should undergo preliminary flotation for fine selective separation, and each fraction should be agglomerated and smelted in separated furnaces. Dust residues must be trapped with the aid of modern, glass cloth filters. Metallurgical combines must be complexes of factories or shops yielding a variety of products and utilizing, without waste, everything of value in the ore. Furthermore, the pastics industry has been rapidly forcing nonferrous metal products out of the consumer market because of their inexpensive and simple production, their toughness and anticorrosive properties, and a number of other qualities which make them irreplaceable, even though they first appeared in the role of "ersatz". The nonferrous metals are not being replaced by plastics, to which the future belongs.

At the end of the first half of the 20th century automobile exhausts resulting from liquid fuel came into being as a serious contaminant. For example, in the USA the number of automobiles increased from 10 million in 1920 to over five times that number in 1950, an increase of 40 million in 30 years. In cities the automobile is a source of various harmful and unpleasant waste products, such as carbon monoxide, hydrocarbons, aldehydes, resin compounds (some of them carcinogenic), products of the breakdown of antiknock agents, which contain finely dispersed vapor of lead oxide, etc.

The most threatening factor in air pollution in the 20th century is the rise of photochemical fog, which is entirely new in man's external environment. Such fog, or "smog" as it has been called, was first observed in Los Angeles in 1945, where by 1950 it had become a serious problem. Smog is formed as the result of photochemical reactions occurring in open air during periods of sunshine. These reactions originate with the organic substances present in automobile exhausts, principally the pentane and hexane olefins with nitric oxides as a major contributor, with the resulting formation of ozone. In the presence of light and hydrocarbons, nitrogen peroxide is converted back to nitric oxide, and the reaction continues as long as there is sunlight. Ozone also reacts with the olefins. As a result of complex and diverse reactions that generate free radicals, ozonides, and peroxides, complexes of organic materials are formed in the atmosphere which are highly active chemically, causing irritation of the mucous membrane of the eye accompanied by lacrimation, loss of visibility, damage to plants, etc. Lacrimation caused by photochemical smog is the most frequent complaint of the population. No successful persistent attempts have been made to determine the connection between smog and its effects. It is possible that each effect is caused by a different chemical agent. A previously unknown compound, peroxyacetylnitrate was isolated from the air, but it had not been established that it was an active component of the Los Angeles smog. However, it has been demonstrated that photochemical fog arose from air contaminated by exhaust gases particularly in the presence of temperature inversion. Smog formation in Los Angeles appears to be the result of unique conditions prevailing there: the absence of public transportation, use of private individual automobiles in the millions, the frequently occurring anticyclone weather, and the constant intense solar radiation are some of such conditions. Recently smogs have appeared in other USA cities including San Francisco, Washington, New York, etc. This atmospheric condition has been gradually spreading over other USA cities. It is reasonable to assume that the effect of photochemical smog in addition to discomforting and disabling effects caused more serious pathologic manifestations, frequently of a chronic nature. The universal rise in automotive traffic progressively enhanced the harmful effects of exhaust gases so that the appearance of analogous photochemical fog phenomena can be expected to appear in other countries. Soviet health authorities should regard this with the consideration it deserves.

The A. N. Sysin Institute of General and Municipal (Public) Hygiene of the USSR Academy of Medical Sciences has initiated preliminary surveys in this direction. Chemist V. A. Popov has perfected the phenolphtalein method used in the USA to determine oxidizers in the air. Tentative investigations in Moscow, Baku, and Batumi have shown that during the summer, i.e., when solar radiation is at its maximum, the products of photochemical transformations of exhaust gases can be discovered in the air of the above-mentioned cities. Although the concentration of the oxidizers is only about 12 percent of those in the air of American cities, they rise close to the critical tolerance established by the California standards. The concentration of oxidizers increased with the number of automobiles, and unless effective measures are taken to reduce the discharge of exhaust gases, cities in the USSR will come to experience smog conditions similar to those occurring in the USA. The universal use of automotive transportation, its rapid and progressive growth in the number of auto vehicles, the discharge of poisonous pollutant components into the air of populated areas, the susceptibility of the components of these gases to undergo diverse photochemical conversions in which highly reactive free radicals participate point to atmospheric pollution by automobiles as an oncoming threatening danger from the viewpoint of modern hygiene. In this connection it must be kept in mind that exhaust gases contained other ingredients, such as lead aerosols, carbon monoxide, carcinogenic substances, etc. This leads sanitary hygienists to the conclusion that air pollution by automobile exhausts constitutes a problem equal in its urgency to that of air pollution caused by industrial combustion, with which hygienists throughout the world occupied themselves earlier. This problem of air pollution by automobile exhausts must occupy a leading place in world's scientific and sanitation efforts. There are means by which this problem can be solved by central regulation of center transportation, proper maintenance of vehicle operation, extending public transit beyond the city limits, converting automotive engines to condensed gas, and converting automotive gasoline to electric engines. Research in the direction of such developments should be conducted on a great scale by engineers and other scientists engaged in solving problems in interstellar space. In this connection it should be noted that radioactive atmospheric air pollution is a specialized field which is intimately connected with international politics is excluded from this discussion. The attention here will be centered on such phases of the problem which deal with the developments in advanced industrial chemistry, which brought into being the most acute and burning hygienic questions of our times.

Chemistry has played an important role in hastening the rise of the Communist society. The 23rd Congress of the CPSU examined the rapid developmental rates of the chemical and petroleum industries. Chemistry and its products extend into all aspects of manufacture and the total life of the population. The manufacture of raw materials, intermediate products, and finished articles from synthetics promises to become one of the most widespread, complex, and massive sources of environmental pollution, especially of the air, unless hygienists, chemists, and engineers investigate these compounds for their characteristics, their biological effects, and their danger to man; learn to isolate them by specific and highly sensitive analytical methods; and prevent their appearance in the atmosphere in concentrations that have a deleterious effect on man.

Environmental pollution has become a universal problem. In March 1963 the World Health Organization in Geneva held a special international symposium on microchemical pollution of the environment. According to this symposium, hundreds of new compounds are synthesized throughout the world each year, many of which find practical application and become a source of environmental pollution. It would be very time-consuming to list the areas in which the new chemical compounds find application, among which are fertilizers, pesticides, defoliants, plastic articles used in machine building, home construction, and civic works; new fibers for clothing, footwear, and other daily usage, packaging, cleaners, detergents, adhesives, synthetic medicines, vitamins, fats, antioxidants; liquid fuel, additives, etc. Most of these organic compounds have never been investigated for their toxicological and physiological properties, nor have means been devised for detecting them in complex mixtures and low concentrations. The possibility of their combined effect, and of their spontaneous change in the open air in some instances into carcinogenic substances and teratogenic effects, complicates the problem considerably. In addition, engineers, builders and consumers ask for data on the harmfulness of new substances which will be used by workers on the job, the inhabitants near the plants, and the millions of consumers of the new chemistry throughout the USSR. For this reason the new developing chemistry must be the principal concern of hygienists, particularly of those who deal with atmospheric hygiene. The problems are complex and may appear insoluble. Therefore, all available resources must be mobilized, the sequence of operations must be strictly planned, and manpower must be properly mobilized, and all available auxiliary resources must be directed toward strengthening the scientific teams which have already been organized and approved. It is easier to achieve a breakthrough where qualified personnel, modern equipment, and an experimental background have built up than where all these have to be created anew. Therefore all resources should be directed toward reinforcing already existing scientific centers for the study of toxicology and chemistry, of new synthetic compounds and the environmental pollutant associated with them, particularly air pollutants. In this respect situations differ in different countries. In the USSR the need is greatest in planning more active investigations of pertinent specific questions, to differentiate, in the first instance, between studies that are absolutely essential for immediate practical results and those of more remote interest. Students working for college degrees should be assigned problems raised by modern chemical industries, especially those interested in the development and production of new synthetics. Results obtained should be published without undue delay so that hygienists and other health authorities could study them with all possible sense of priority. This applies particularly to organic compounds and substances which are highly volatile, have a definite odor, and irritate the mucous membranes, especially of the upper respiratory tract. They can be neutralized in the following ways:

1. Burnoff in special furnaces. Unfortunately, because of the low concentrations of contaminants and the inadequate heat of combustion, fuel has to be expended to burn them. The drawback of this method is the destruction of valuable organic materials that could be extracted and used in the national economy. On the other hand, where these compounds cannot be rendered harmless in any other way, this is an appropriate method.

2. Ordinarily, compounds of this type can be easily condensed and, therefore, lend themselves to low-temperature separation. After the basic mass has been condensed, the remainder, which has no value, can be burned.

3. These compounds can also be absorbed, with subsequent desorption or elution of the products and regeneration of the filters. This method of recuperation is widely used in practice to avoid discharges of organic compounds. By combining these three methods it is possible to achieve satisfactory disposal of the most varied discharges of synthetic chemistry.

For materials with a low activity threshold, means must be found of replacing them with less toxic compounds or of introducing a technology based on essentially different methods, i.e., methods which did not require toxic materials. For example, the high-temperature heat conductor dynel, which is widely used in synthetic chemistry and pollutes vast areas in the manufacture of synthetic fibers, can be replaced by electric heating. The appropriate technical institutes need to devote considerably more attention to developing this advanced method in their planning.

By systematizing the different types of the air-pollution problems related to plastic chemistry industries, it is possible to arrive at a means of lessening or abolishing their pollution effects by burning residual discharges or other suitable means based on the following two prerequisites:

1. Hygienists and toxicologists must actively participate in the

activities in the work of industrial research institutes engaged in developing industrial synthetic products.

2. Maximum support must be given to studies of the biological and toxicological effects of new chemical compounds introduced into production, so that their critical concentration tolerances (MAC) could be determined as soon as possible, and specific and highly sensitive methods of detection can be developed.

The foregoing makes it clear that the problem of air pollution has become considerably more complex and difficult in its century-long history, and has given birth to new questions different from the ones which prevailed during the first half of the present century.

The principal sources of air pollution now are industries of the new synthetic chemistry and automotive exhausts. The present volume is devoted to discussions of practical problems caused by automotive exhausts.

NEW EXPERIMENTAL MATERIAL FOR THE DETERMINATION OF MAXIMUM ALLOWABLE CONCENTRATION OF HEXAMETHYLENEDIAMINE IN THE AIR OF INHABITED AREAS

From the A. N. Sysin Institute of General and Municipal Hygiene of the USSR Academy of Medical Sciences

Soviet and foreign literature dealing with the biological effects of hexamethylenediamine (HMD) and on its effects on man is limited. Reported studies were made using small numbers of experimental animals, and high concentrations of the investigated substance. The most pertinent data found in the literature relative to HMD effects on the respiratory tract were found in two experimental papers published by V. S. Filatova, V. A. Russkikh, He. V. Chernet, and T. G. Lapina in 1951, and by A. P. Martynova in 1957. Analysis of HMD biological effect in acute and chronic inhalation experiments has shown that it is a toxic substance having a general resorptive action resulting in the constriction of the capillary and small blood vessels and in dystrophic changes in the lungs, liver, kidneys, spleen, heart, brain, spinal cord, stomach, and intestines. HMD depresses effect on the central nervous system, lowers arterial pressure, and changes the morphological composition of the blood. According to Martynova, this toxic effect occurs at concentrations of 1 mg/m^3 and higher. No lower concentrations have been studied. A literature survey conducted by Paton in 1957 indicated that the toxic action of HMD rested in the capacity of mono- and diamines and other related chemical substances to liberate bound hystamines and other related biologically active substances. Diamines and amines of high carbon atom numbers possessed this effect in the highest degree.

In a paper published in 1964 dealing with the reflex action of HMD in man, the present author proposed 0.001 mg/m^3 as the maximum allowable single-exposure concentration in atmospheric air. The present paper presents results of animal inhalation tests of low HMD concentrations 24 hours daily for 3 months. In this study 60 white male rats were divided into four equal groups. Animals of Group 1 inhaled air containing HMD in concentrations of 1 mg/m^3 or the maximum allowable for industrial premises. Animals of Group II inhaled air containing HMD in concentrations of 0.64 mg/m^3, or the maximum single-exposure allowable concentration in atmospheric air. The animals were checked during inhalation, also for a month before and after inhalation exposure by the following tests: weight, reticulocytes, leucocytes, and eosinophile counts; luminescence and phagocytosis; Vi-antibody titre after immunization against typhoid fever; motor chronaxy of the antagonist muscles; and rate of coproporphyrine elimination. After the inhalation period, internal organs were studied for macro and microhistological changes.

Table 1 presents the data obtained from determining the HMD concentration on a daily basis.

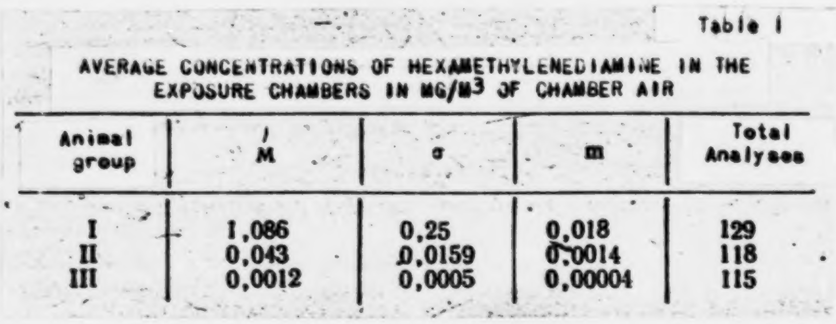

Table I

AVERAGE CONCENTRATIONS OF HEXAMETHYLENEDIAMINE IN THE EXPOSURE CHAMBERS IN MG/M^3 OF CHAMBER AIR

Animal group	M	σ	m	Total Analyses
I	1,086	0,25	0,018	129
II	0,043	0,0159	0,0014	118
III	0,0012	0,0005	0,00004	115

Animals were weighed once every 10 days. Animals of Group I lost 5.7% of their preexperimental weight in the course of inhalation exposure, as shown by graphs presented in Figure 1.

The other two groups presented no differences in weight from the controls. In her experiments Martynova also noticed that during HMD

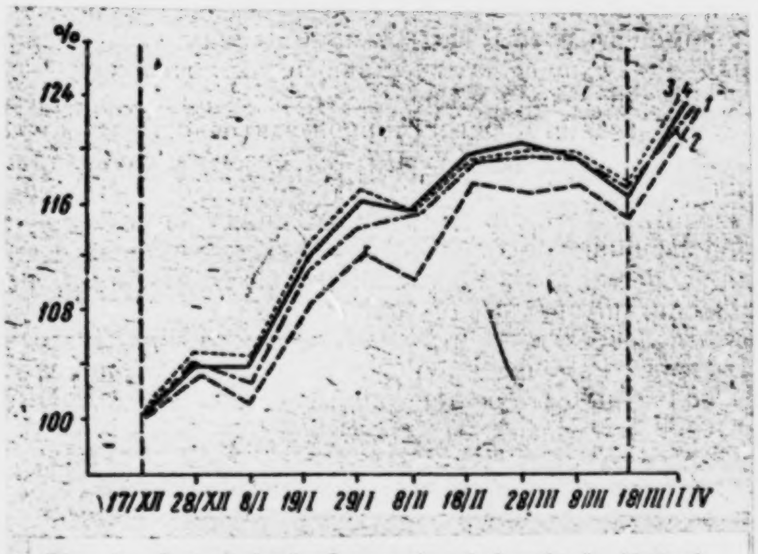

Fig. 1. Body weight of experimental animals through the chronic experimental inhalation period of low hexamethylenediamine concentrations.
1 – 1 mg/m³; 2 – 0.04 mg/m³; 3 – 0.001 mg/m³;
4 – Clean air

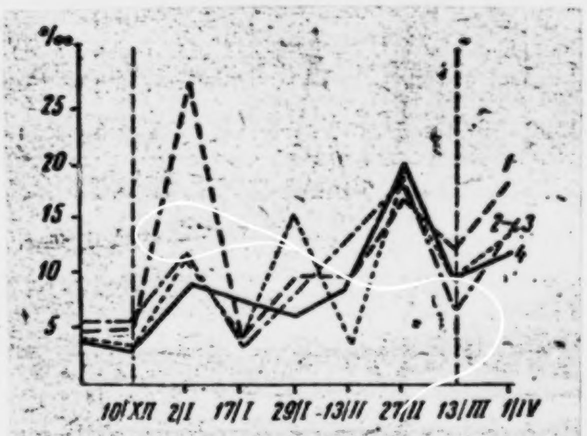

Fig. 2. № of reticulocytes in the blood during the period of chronic experimental inhalation of low hexamethylenediamine concentrations
1, 2, 3, and 4 same as in Fig. 1.

inhalation by white rats 6 times daily for 6 months in concentrations of 10 and 1 mg/m^3 the experimental animals lost weight as compared with the controls.

Reticulocyte counts made by the A. P. Yegorov method showed that only 1 mg/m^3 HMD concentration induced after two weeks a statistically reliable (R = 0.99) increase in reticulocyte count as compared with the control group. However, two weeks later the count returned to its normal level, as shown by curves in Figure 2.

The leucocyte count, determined by the N. M. Nikolayev method (1954), was practically the same as of the control group.

It has been established by V. N. Chernigovskaya, A. Ya. Yaroshevskii (1953, 1957), K. Kh. Kyrche (1956), et al, that the eosinophile level, like that of other formed blood elements, depended on the functional state of the brain. Investigators such as Thorn (1953), I. A. Eskin, G. M. Vidavskaya (1956), Aschekenasy (1957); I. A. Eskin, N. V. Mikhaylova (1953), et al., found that the hormonal system, consisting of the anterior pypophyseal lobe and the adrenal cortex, that controls the hypothalemus, affected the eosinophile content of the peripheral blood. Recent pathological investigations pointed to functional shifts in this hormone system which effected changes in eosinophile counts in man and in experimental animals when toxic substances were administered in concentrations present in the air of industrial workrooms (Ye. I. Spynu, 1962; L. E. Gorn, 1963; et al.).

It has been shown that an allergic reaction raised the eosinophile count in the peripheral blood, as a protective reaction, reflecting the eosinophiles' capacity to reduce the stress of the allergic reaction by inactivating the hystamines, heparines, and other biologically active substances the number of which in free states increased sharply, as was shown by I. P. Lepner, Ye. S. Brusilovskiy, 1961; L. D. Grishpun, 1962, etc. It is important that the effect of HMD on eosinophile count in the peripheral blood under the conditions of this experiment be clearly understood. Using the S. M. Bakman method (1958), the present author made absolute counts during the first month of inhalation exposure once a week; and thereafter, once fortnightly, totaling 223 blood analyses.

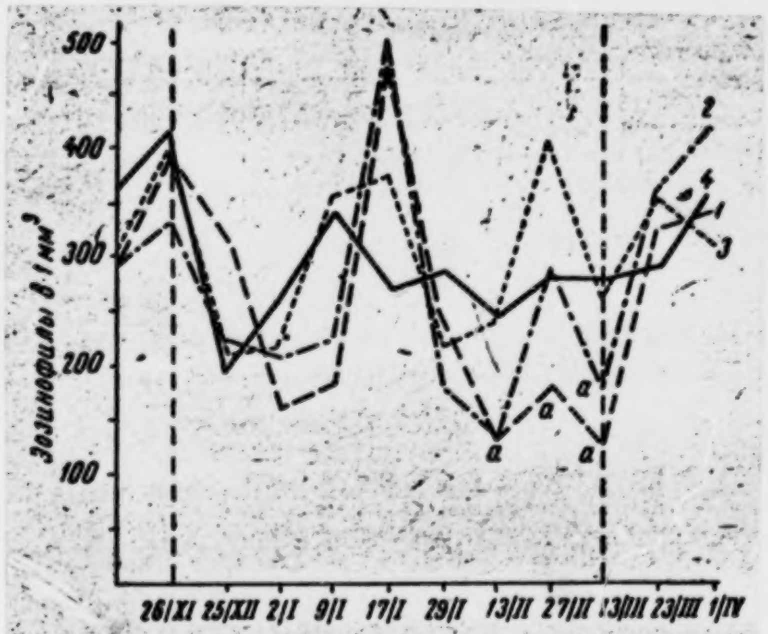

Fig. 3. Absolute eosinophilic count in the blood of experimental animals during period of chronic inhalation of low hexamethylenediamine concentrations. 1, 2, 3, and 4 same as in Fig. 1.

Fig. 3 shows that after a week of inhalation exposure all groups manifested a drop in eosinophile counts, and at the end of the second week the rats of Group I reacted most profoundly; however, in comparison with the controls they evidenced no statistically reliable differences. For technical reasons the interval of inhalation exposure was interrupted for two days (15 and 16 January) at the end of the first month; 14-16 hours after its renewal a sharp increase was noticed in the amount of eosinophiles in the blood of animals of Groups I and II, particularly in rat No. 3 of Group I and rat No. 4 of Group II, whose counts before-and-after were respectively 474/1313 and 200/837 per m^3 of blood. Subsequently the eosinophile count in the animals of the first two groups dropped to a constant level at the end of the experiment with statistical R = 0.95. In these groups the eosinophile counts dropped more than 50% in comparison with the initial counts, pointing to compensatory functions (S. M. Bakman, 1960; et al.), inherent in the hormonal system (anterior hypophyseal lobe, adrenal cortex), under HMD in concentrations of 1 and 0.4 mg/m^3. Animals of Group III had an eosinophile count of 0.0001 mg/m^3, which was close to that of the controls. In other words, the tests indicated a non-specific reaction of the organism to the negative effects of microconcentrations of atmospheric contaminants. Many authors (M. N. Meysel', V. A. Sondak, 1956; S. S. Sergel', A. A. Klimenko, 1957; M. Ya. Khodas, 1959; Ye. B. Zakrzhevskiy, L. G. Vasil'yeva, 1936; et al.) have shown that the nature of leucocyte luminescence during vital staining with acridine orange changed in blood diseases, or in ionizing radiation, the leucocytes fluorescence changing from green to yellow, orange, and red as a result of cellular physiochemical changes.

A. D. Semenenko (1963) and P. G. Tkachev (1963) observed a change in leucocyte luminescence following the inhalation of low aniline concentrations. M. I. Gusev and K. H. Chelikanov (1963) noted the same for amylene. For this reason the present author used acridine orange in 1:50,000 concentrations in a physiological solution. 0.02 ml of blood was taken from the animal's tail vein and placed in a test tube containing 0.18 ml of the acridine solution. Then a single drop of fluorochromed blood from the test tube was placed under a microscope in the usual manner. A Reichert microscope was used for immediate examination at 5 x 60 magnification. One hundred leucocytes were counted in each specimen, and the percentage changes of leucocytes with illumination were calculated. Altogether, 182 blood samples were thus examined.

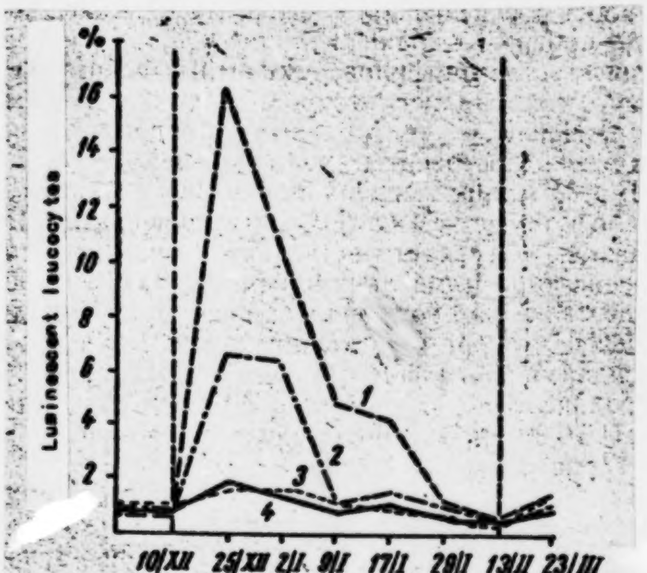

Fig. 4. Percent of luminescent leucocytes during the period of chronic inhalation of low hexamethylene diamine concentrations
1, 2, 3, and 4 same as in Fig. 1.

Fig. 4 shows that after a week's inhalation the Group I animals' leucocyte illumination percentage rose and then, toward the beginning of the second month, returned to the initial level. In some animals the changed cells reached 21-25%. This increase was less pronounced in animals of Group II (9.11% in isolated cases), and returned to normal after 3 weeks inhalation. This phenomenon was noted also by M. I. Gusev and K.N. Chelikanov (1963). The increase in the % of leucocytes with varying illumination, observed in the case of animals of Groups I and II, is statistically reliable (R = 0.05 and 0.99). Yellow and orange tones predominated, with red appearing only occasionally. Data of Group III differed little from those of the controls.

The protective and adaptive functions of the organism depend upon its general physiological reactivity, which in turn is a function of many factors in relation to the external environment (physical, chemical, biological, alimentary, etc.) which, in acting upon the organism, can alter its resistance and susceptibility (A. D. Ado). The immunobiological reactivity of the organism can serve as a clue to its general biological reactivity, which in turn can serve as a clue to specific and general resistance.

Studies in occupational pathology (T. V. Rasskazova, 1958; A. P. Volkova, 1959; V. K. Havrotskiy, 1960; A. I. Pakhomychev, 1960; A. F. Stoyanovskiy, 1961; et al.) indicated changes in organism's immunobiological

reactivity under industrial conditions. The same is true of experimental animals exposed to inhalation of poisonous substances for prolonged intervals (SO_2, CO_2, lead, tetraethyl lead, CCl_4, nitrobenzene, benzene, anilene, etc.). Depression of phagocytosis and of antibody formation, under the effects of harmful substances in concentrations at or exceeding the MAC levels for indoor air under working conditions has been recorded, which induced the present author to study the possibility of reducing the immunobiological indices during chronic HMD microconcentrations inhalation.

In the study of phagocytosis a 24-hour culture of staphylocuccus in a concentration of 500 million per ml was used. Equal quantities (0.06 ml) of blood, in a 2% sodium citrate solution, and a microbe suspension were used. The phagocytosis reaction lasted 20 min at 37°C. After centrifugation a leucocyte smear was made and stained by the Bimza-Romanovskii method. One-hundred neutrophiles were counted along with the percentage of active leucocytes (extent of phagocytosis) and the phagocytic index was determined. This approach was suggested by V. S. Gostev and associates in 1950. To determine the phagocyte activity of the leucocytes 204 blood analyses were made, the first two a week apart, the remainder every two weeks.

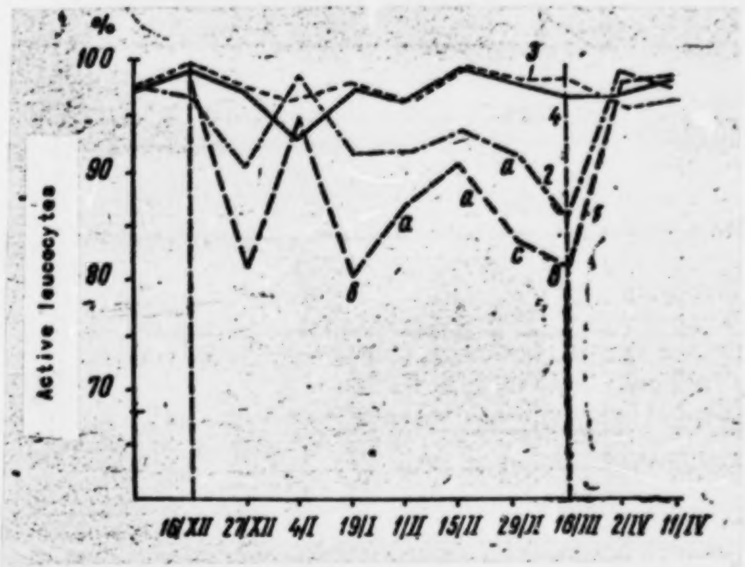

Fig. 5. Phagocytic leucocyte activity on the basis of extensive indicator in experimental animals during chronic experimental inhalation of low methylenediamine concentrations

1, 2, 3, 4, same as in Fig. 1.
Degree of reliability – a – 96%, b – 99%, c – 99.9%

The results indicated that the percent of active leucocytes in animals of Group 1 (1 mg/m^3) dropped sharply after one week's inhalation of HMD, while after two weeks it began to rise to a level above that of the control group. This was followed by a new depression in phagocytic activity which persisted to the end of the inhalation period. The most pronounced change occurred in rat No. 14 of Group I, pre-inoculation phagocytic index of which was 99.3-100; after a week of inhalation it dropped to 55.1, rose again to 92.9 after two weeks, and finally remained stationary at 79.3. At the end of the inhalation period the percent of active leucocytes in Group I animals rapidly returned to the background level. However, such statistically reliable rosults are not less pronounced than the changes noted in Group II animals (0.04 mg/m^3).

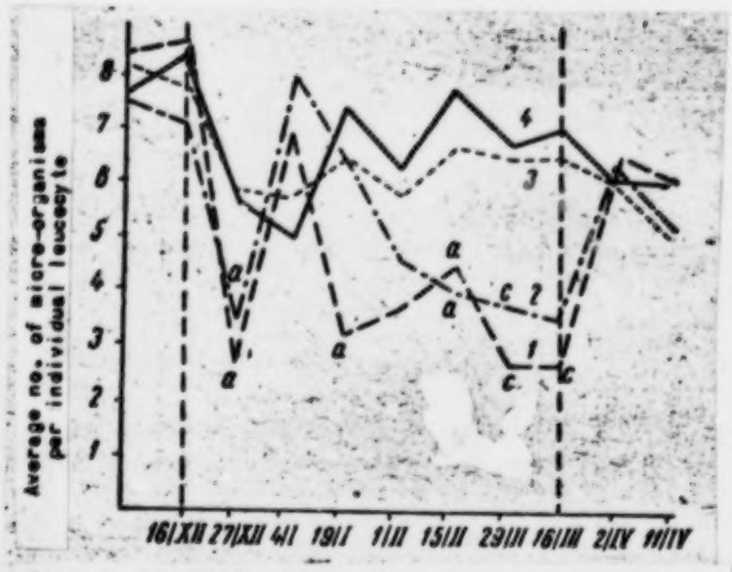

Fig. 6. Phagocytic leucocyte activity on the basis of intensive indicator in experimental animals during the period of chronic experimental inhalation of low hexamethylenediamine concentrations
1, 2, 3, and 4 same as in Fig. 1.

Fig. 6 shows that the intensity of the phagocytic process also underwent analogous and reliable changes in the animals of Groups I and II. Animal No. 14 in Group I again exhibited the most pronounced changes. Prior to inhalation the phagocytosis index was 7.4-7.9, while after a week it dropped to 1.3. After two weeks it rose to 5.3, then dropped again to 2.5, where it remained, with several fluctuations, to the end of the experiment. Changes in phagocytic index in animals of Group III (0.001 mg/m^3) did not differ substantially from that of the controls. On the basis of the above it can be stated that the dynamics of the phagocytic process in all animals of Groups I and II followed a generally regular pattern of depression. A rise in phagocytic activity in the second inhalation week following a sharp depression indicated that the phagocytic process had undergone phase changes during chronic inhalation of HMD by rats exposed to concentrations of 1 and 0.04 mg/m^3.

Experimental animals were vaccinated with Vi typhoid fever vaccine manufactured at the Moscow Scientific Research Institute for Epidemiology and Microbiology. Since nothing was found in the literature describing procedures for immunizing rats, the investigation was of an exploratory character. Vaccination was initiated a month after HMD inhalation, when changes could be expected in the organism's reactivity. The vaccine was injected subcutaneously into the hind leg paws. The first injection consisted of 200 million typhoid fever bacilli and 0.04 mg of typhoid fever Vi-antigen. After a week the second and following injections consisted of 300 million typhoid fever bacilli and 0.06 mg of Vi-antigen. After standing a day, 0.5-0.6 ml of blood was taken from the rats' vein and the serum was allowed to separate from the clot at 34° C. The Vi-antibody titre was determined by the passive hemoagglutination reaction recommended by N. A. Kraskina and N. M. Gutorova in 1962. Before the vaccine was administered the Vi-antibody titre in the experimental animals was practically nil. A week later Vi-antibody titre in the Group I animals was lower than in the controls, not even a trace of it was found in rat No. 12 of Group I. A somewhat less expressed phenomenon was noted in rats of Group II.

A statistically reliable difference (R=0.95) in the Vi-antibody titre was noted only in animals of Group I a week after the second inoculation. After injections at the end of the inhalation exposure and during the recuperation period, the titre did not differ significantly from that of the control group. Animals of Group III which inhaled the lowest HMD concentration (0.001 mg/m^3), manifested no titre-levels differing from the controls throughout the test period.

Studies of coproporphyrin elimination rate via the urine were conducted using an SF-4 spectrophotometer as recommended by M. I. Gusev, Yu. I. Smirnov, 1960. Urine was collected daily in special containers every 10 days from rats of each group. Before inhalation exposure and during the recuperation period, 3 examinations were made for each rat, and 9 during the inhalation exposure. Fluctuations in the rate of coproporphyrin elimination via the urine of Group I animals during the HMD inhalation period pointed to a tendency toward reduction of its excretion rate on some days of the first half of the experimental inhalation; however, the difference was not statistically significant. A reliable difference was observed during the recuperation period, which was related chiefly to a drop in the control animals. No substantial differences from the control animals were found in animals of Groups I and II.

Results of motor chronaxy (ratio) tests showed that disturbance of the normal ratio occurred in animals of Groups I and II a month after inhalation exposure was initiated; it persisted at this level with certain fluctuations for the next 40 days of the inhalation exposure period (Fig. 7), after which the normal ratio was restored. A statistically reliable difference was observed in animals of Groups I and II on the 50th day of inhalation (R = 0.99 in both groups). In animals of Group III the chronaxy ratio varied within the limits of physiological fluctuations.

Internal organis of 26 rats were examined by sacrificing them immediately after inhalation, and 19 animals were sacrificed 2 months later to study latent effects.*

Disection of Group 1 animals (1 mg/m^3) revealed a fatty depletion of the hypodermic tissue. Examination of the liver disclosed venous plethora (Fig. 8), vascular wall plasma infiltration and perivascular lymphoidal-hystiocytic nodules. The liver glycogen content was stable, with a slight reduction in isolated cases.

The miocardium showed the presence of individual or groups of unevenly stained fibers on the background of venous plethora. The transverse striations and nuclei were particularly marked. Only plethora and lymphoidal infiltration along the tubules were found in the kidneys. Lipides were discovered in all three zones of the intestines.

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*Doctor of Medical Sciences V. P. Osintsevaya supervised the pathomorphological examinations, which were conducted at the Pathomorphology Laboratory of the A. N. Sysin Institute of General and Municipal Hygiene of the USSR Academy of Medical Sciences.

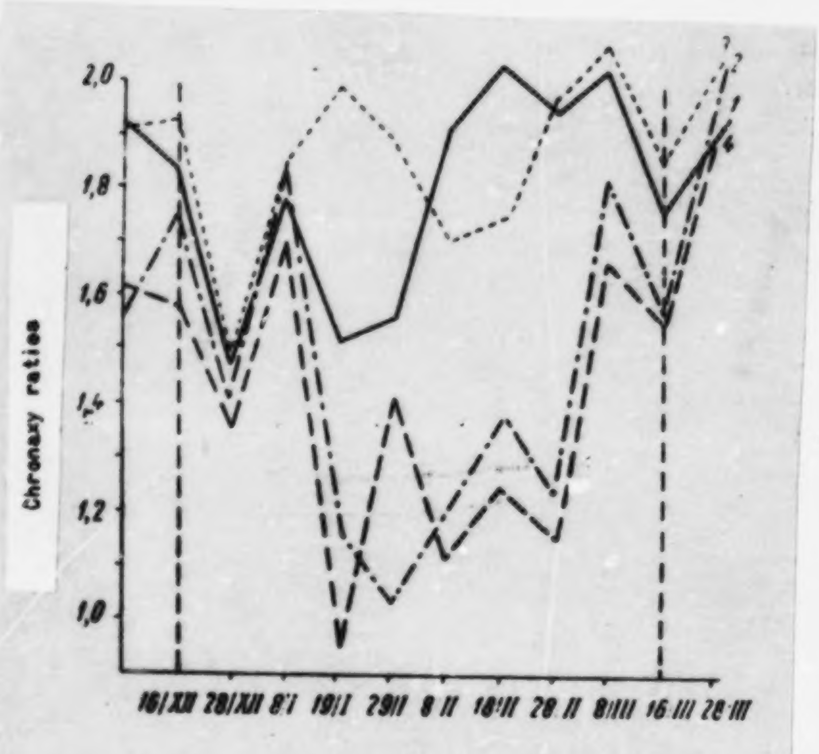

Fig. 7. Muscle antagonist motor chronaxy ratio in experimental animals exposed to chronic experimental inhalation of hexamethylenediamine concentrations
1, 2, 3, and 4 same as in Fig. 1.

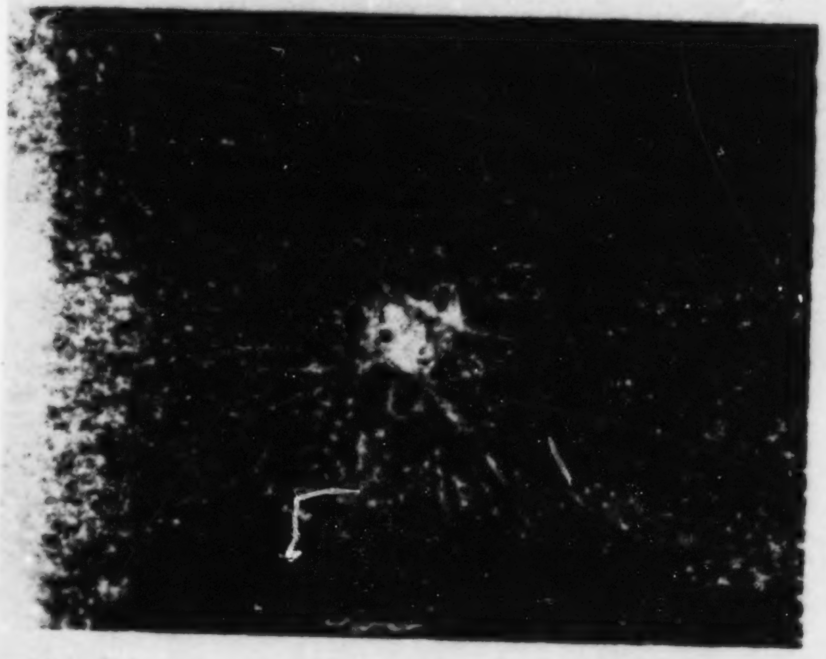

Fig. 8. Liver section of animal No. 2 of Group I hematoxylin stained. Magnification 10 x 10.

The capillary brain network of Group I animals was slightly distended. Cells with vacuolated cytoplasm, with dispersal or thickening of the Nisslevskiy matter blocks and very occasionally with cromatolysis were found in the cerebral cortex and subcortical nodes. Cells with pericellular edema were also encountered (Fig. 9).

The liver, miocardium, kidneys, and brain of the Group II animals (0.04 mg/m^3) showed changes similar to those found in Group I, but in a less pronounced degree. The quantity of intestinal lipides suffered a reduction.

Examination conducted after 2 months of inhalation exposure showed that animals of Groups I and II exhibited a purulent penumonia of a rather severe intensity which rose with increase in the HMD concentration.

Organic changes in animals which inhaled HMD in concentrations of 1 and 0.04 mg/m^3, manifested a monotypical character both in immunized and non-immunized rats. Follicles were found more often in the spleen of the immunized animals than in the nonimmunized, and

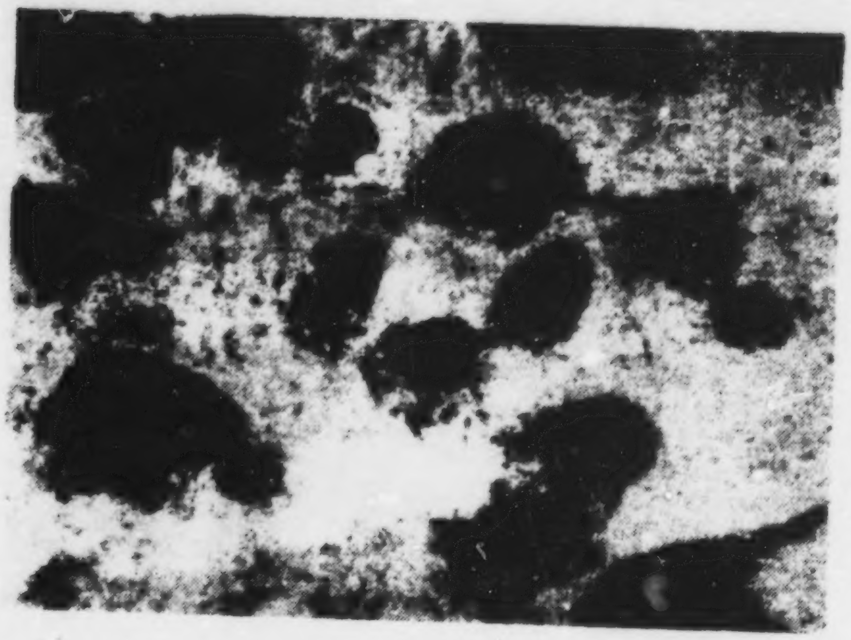

Fig. 9. Brain section of animal I of Group I, stained by Nissel method. Magnification 90 x 15.

their size was considerably larger. The number of plasmatic cells in the immunized rats increased markedly, in connection with which the formation of antibodies was observed. The morphology of the immune process in animals of Groups I and II (1 and 0.04 mg/m^3) was less pronounced than in the other two. Group III animals (0.001 mg/m^3) exhibited no morphological changes from those observed in the controls.

Results of the experiments point to the unfavorable effect of small HMD concentrations (1.0 and 0.04 mg/m^3), most strikingly in the loss of weight in Group I animals, and in the increased number of reticulocytes. Animals of Groups I and II also exhibited an eosinopenic response and an increase in the percent of leucocytes with a change in the luminescence.

The results also show that such low concentrations of HMD can affect the state of the organism's immunobiological reaction inducing depression of the phagocytosis processes, of antibody formation, and other immuological processes. Animals of Groups I and II also negatively affected the normal ratio of antagonistic-muscle chronaxy, as well as pathomorphological changes in the internal organs.

The HMD concentration inhaled by animals of Group III (0.001/m^3) was the only one having no effect in any of the tests.

CONCLUSIONS

CONCLUSIONS

1. Continuous inhalation of HMD in 1 mg/m^3 concentration for a period of three months unfavorably affect the organism of experimental animals inducing loss of body weight, raising the reticulocyte count, producing eosinopena, increased leucocyte count, lowering the phagocytic index and the rate of Vi-antibody productions, upsetting the normal motor chronaxy ratio in the antagonistic muscles, and inducing pathomorphological changes in the internal organs.

Similar but milder changes are caused by an HMD concentration of 0.04 mg/m^3 with the exception of loss of body weight and the number of reticulocytes.

2. In 0.001 mg/m^3 concentration HMD administered under identical conditions has no effect on the organism of the test animals.

3. The average daily maximum permissible concentration of HMD in atmospheric air in inhabited areas can be the same as was previously proposed for the maximum single-exposure concentration, i.e., 0.001 mg/m^3.

HYGIENIC BASIS FOR THE DETERMINATION OF THE ALLOWABLE CONCENTRATION LIMIT OF DIVINYL IN ATMOSPHERIC AIR

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Divinyl (butadiene-1, 3) is a basic monomer used in the production of synthetic rubber. As a diene hydrocarbon with two conjugate double bonds it polymerizes easily and lends itself to the manufacture of various kinds of synthetic rubber. Under ordinary conditions divinyl is a gas having a most unpleasant odor and a density of 1.87; at a temperature of minus 4.5°C divinyl condenses into a colorless, transparent, volatile liquid. It is weakly soluble in water, dissolves easily in acetone, benzene, and amylacetate (A. A. Petrov, 1953) and less so in ethyl alcohol (P. Vatsulik, 1960).

V. A. Pakrovskiy (1955), V. S. Freydlin (1935), L. F. Larionov (1954), E. N. Levina (1948), Henderson, Haggard (1930) and others characterized high divinyl concentrations as narcotic and a mucous membrane and hemogenic organic irritant. The maximum allowable concentration for industrial premises is 100 mg/m^3.

Disorders of the central nervous system such as rapid fatigue, headache, drowsiness have been observed among divinyl workers. Rhinitis, larngitis, pharyngitis, conjunctivitis, changes in the composition of the peripheral blood, dyspeptic phenomena, drop in arterial pressure, etc. were recorded by S. B. Glauberman (1957), L. I. Gefter, Ye. D. Shul'man (1935), S. D. Polyak, N. I. Pil'man, A. I. Zaretskaya, and S. M. Kleyn (1935), A. A. Yegunov (1940), E. N. Levina (1948), A. A. Orlova and G. N. Mazunia (1960), L. F. Larionov (1954). Most of the symptoms appeared after contact with divinyl has been discontinued.

Animals suffering of chronic divinyl poisoning usually exhibit slight increase in mucous cells of the epithelium of the bronchi, local microcellular infiltration, emphysema of the lungs, hyperplasia of the bone marrow with an increase in immature forms, an increase of myeloid elements in the red pulp of the spleen, and symptoms of irrigation in the lymphatic nodes. (L. F. Larionov, 1954).

Most of the cited investigators described cases of industrial workers exposed to divinyl in heavy concentrations. No data were found dealing with the effects of low concentrations in atmospheric air. The widespread use of divinyl by synthetic rubber industry prompted the present author to study the effects of divinyl microconcentrations to determine a hygienic basis for its maximal allowable concentration in atmospheric air, which is the purpose of the present paper. The work was conducted under the supervision of Candidate of Biological Sciences M. D. Manit; and a spectrophotometric method for determining microquantities of divinyl under experimental conditions and in atmospheric air was developed, and the optimal parameters for selecting air samples were determined. The method enabled to determine divinyl in atmospheric air under natural conditions in the presence of other synthetic rubber production compounds such as alpha-methylstyrene, isopropylbenzene, butane, and butylenes.

The threshold of odor perception was determined by a generally employed method (V. A. Ryazanov, K. A. Bushtuyeva, Yu. A. Novikov, 1957). However, since under ordinary circumstances divinyl is a highly volatile gas, certain additions to the method were made, related to gas dosing in the system. An experimental mixture of gas and air in the required concentration was made in 40 liter steel cylinders (oxygen or nitrogen) by exhausting them and then filling them with a small amount of gaseous divinyl. After being introduced into the cylinder the divinyl was rarefied and evenly mixed with a large volume of compressed air up to a pressure of 20-30 atm or more. A constant divinyl concentration was maintained when the mixture was taken from the cylinder through a reducing valve. The divinyl concentration in the cylinder was computed; in the gaseous phase it was measured by an SF-4 spectrophotometer. By changing the rate of mixture flow from the cylinder and rarefying it with pure air forced into the cylinders by means of air blowers at a rate of 15-20 l/min, it was possible to reach the divinyl concentration required for inhalation in the experiments. This method of gas mixture feeding insured an even and constant divinyl concentration in the cylinders. By using a reducing valve to change the air-divinyl flow rate from the cylinder, it was possible to obtain the necessary gas concentration in the system. The rate of the gas mixture flow was determined by a rotating flow meter installed below the reducing valve.

Sixteen persons 18 to 36 years of age having no deviations from the normal state of the ear, throat, and nose were used in the odor perception threshold tests. All volunteers were first familiarized with the odor of divinyl. The acrid and unpleasant odor of the gas is easily distinguished and remembered. The divinyl concentration in the cylinders was checked before and after each experiment. Altogether 336 tests were run at divinyl concentrations of 6.0, 5.2, 4.8, 4.4, 4.2, 4.0, and 3.8 mg/m³. Results are recorded in Table 1.

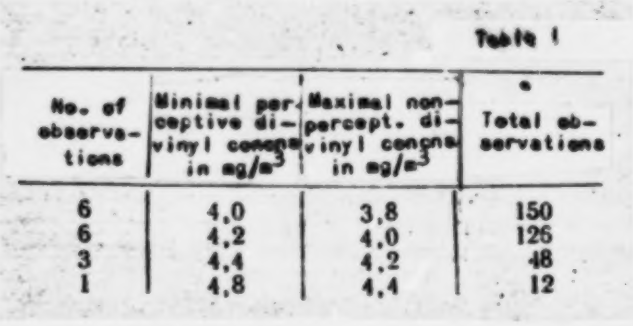

Table 1

No. of observations	Minimal perceptive divinyl concns in mg/m³	Maximal nonpercept. divinyl concns in mg/m³	Total observations
6	4,0	3,8	150
6	4,2	4,0	126
3	4,4	4,2	48
1	4,8	4,4	12

The data show that the threshold as established by the experiment was 4 mg/m³. Effect of divinyl on eye sensitivity to light was studied by the method recommended by the Committee on Sanitary Protection of Atmospheric Air, using 4 persons aged 20-25 with normal vision and odor perceptions. Sensitivity to light studies were conducted after a 10-min disadaptation. The first 4-6 days the volunteer inhaled pure air as described elsewhere; the following days divinyl was introduced into the cylinder in concentrations below the olfactory threshold between the 15th and 20th minute with the subjects seated in a dark room. Test persons were tested on three successive days to determine the effect of the gas in the above cited concentrations.

The first concentration, 4.2 mg/m³ elicited no odor perception response; however, as in the case of 4.0, all testers became more light-sensitive on the 20th minute as compared with pure air. All results were statistically significant.

Two of the volunteers, (P.A.K. and U.A.F.), manifested higher sensitivity at 3.8 mg/m^3, but in only one of these was the increase statistically signigicant. In the remaining cases the sensitivity change was not statistically reliable, even with a certain change in the sensitivity curve. No effect was detected at 3.6 mg/m^3 gas concentration as shown in Fig. 1.

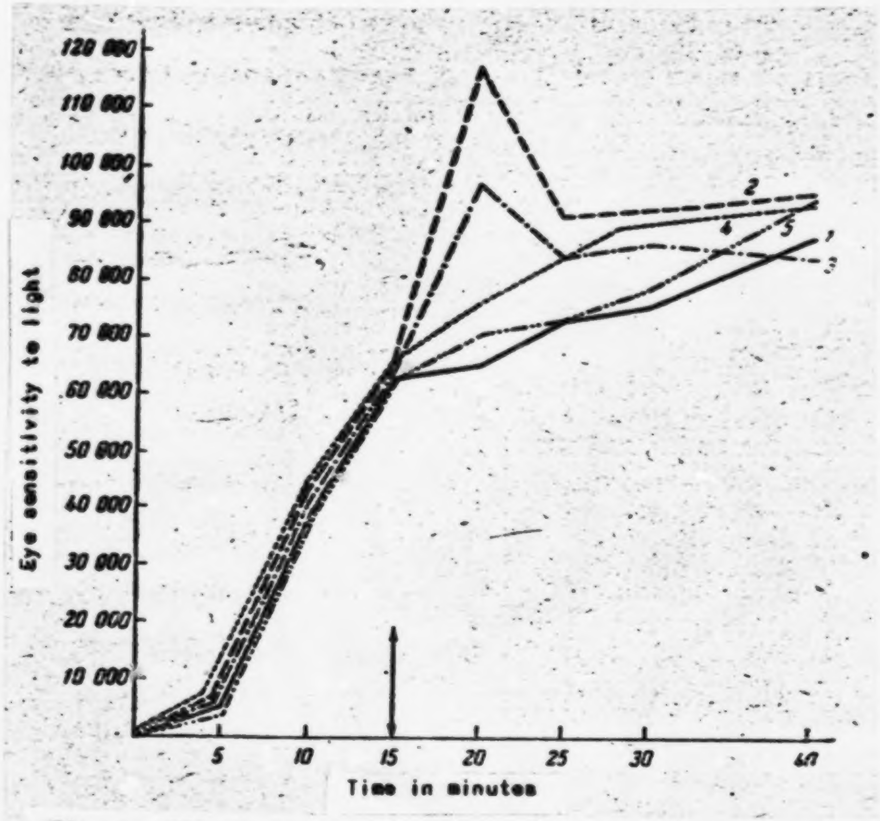

Fig. 1. Effect of divinyl inhalation in different concentrations on eye sensitivity to light of female test person P.A.K.
1 - Clean air; 2 - 4,2 mg/m^3; 3 - 4 mg/m^3; 4 - 3.8 mg/m^3; 5 - 3.6 mg/m^3

The reflex-action threshold for eye sensitivity to light was, thus, 3.8 mg/m^3, which is about the same as that for odor perception.

The central nervous system threshold was determined by generating an electrocortical conditioned reflex as described by K. A. Bushtuyeva, Ye. F. Polezhayev, and A. D. Semenko in 1960 using an electrocencephalograph manufactured by the Kaiser Company,* and 24 test persons. Of these 2 men and 2 women aged 18-30 were selected having a clear alpha-rhythm and well defined synchronization to light. All test volunteers first participated in a similar experiment and in the course of their training gave good, clear encephalogram readings. Tests were made daily at the same hour for each individual.

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*This work was conducted at the Department of Municipal Hygiene of the Central Institute of Physician Training under the direction of Doctor of Medical Sciences K. A. Bushtuyeva.

The divinyl concentrations tested were 3.8, 3.6, and 3.0 mg/m³. At the first concentration two individuals developed a conditioned electrocortical reflex; A. S. achieved this at the sixth session on the first day and at the fifth on the second day. This concentration had an analogous effect on the 3rd and 4th days, but the two lower concentrations did not provoke a reflex in this test person. At the 5-17th sessions G. Kh. developed the reflex at 3.8 mg/m³, had a very slight reaction at 3.6, and none at 3.0. R. P. and G. G. developed a conditioned electrocortical reflex at 3.6 mg/m³ (Fig. 2) and none at concentration of 3.0 mg/m³ (Fig. 3).

Table 2

EYE SENSITIVITY TO LIGHT DURING EXPERIMENTAL DIVINYL INHALATION (ON THE 20TH MINUTE OF INHALATION IN PERCENT OF THE 15TH MINUTE OF INHALATION)

Initials of test persons	Clean air	Mg/m³ of divinyl							
		4.2		4.0		3.8		3.6	
		%	Reliability %	%	Reliability %	%	Reliability %	%	Reliability %
U.A.F.	128	156	99,9	131	99,0	130	75,0	128	No change
S.G.B.	111	129	99,9	116	90,0	110	50,0		
P.A.K.	112	176	99,9	146	99,9	120	98,0	112	No change
D.E.N.	123	222	99,9	128	50,0	121	50,0		

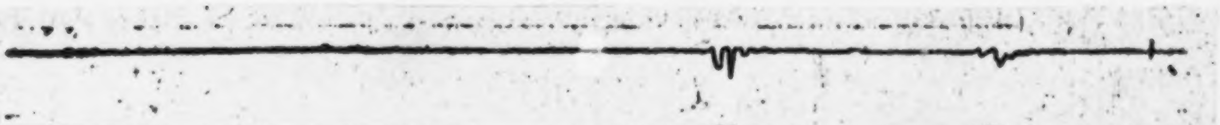

Fig. 2. Electroencephalogram of R. P. Conditioned reflex alpha-rhythm desynchronization on the 8th combination during 3.6 mg/m³ divinyl inhalation; AC – inhalation of investigated gas concentration; BC – switching of light

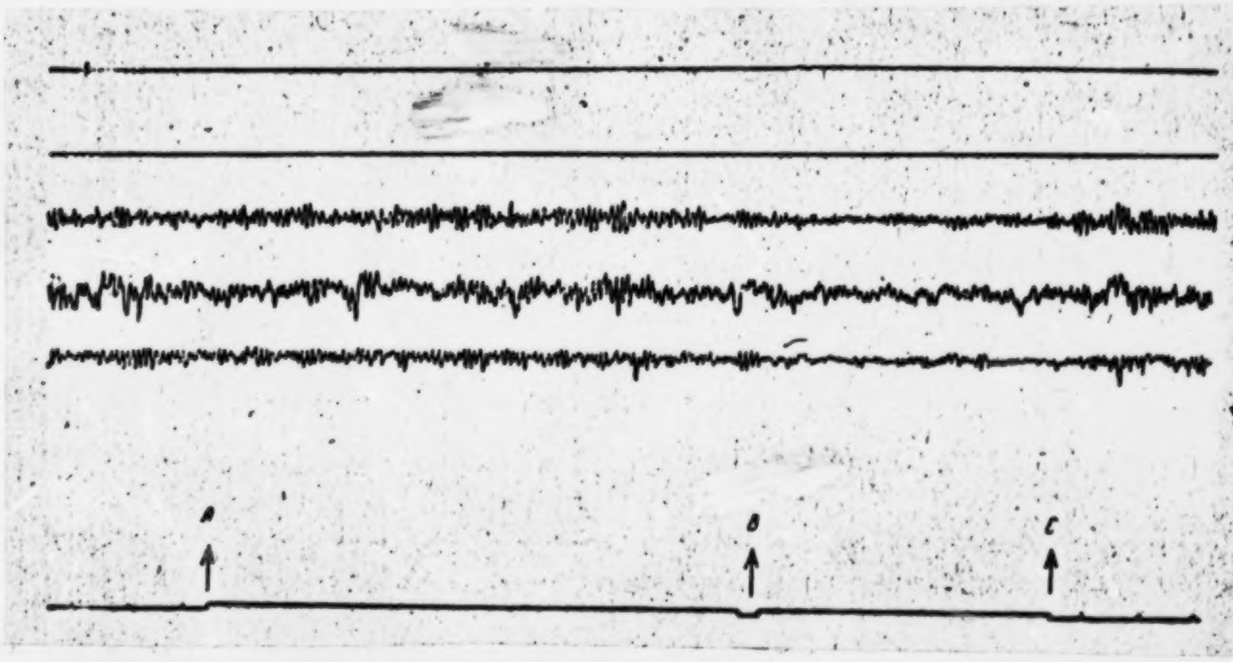

Fig. 3. Electroencephalogram of R. P. No conditioned-reflex alpha rhythm desynchronization during inhalation of 3 mg/m^3 of divinyl. AC and BC same as in Fig. 2.

In other words, a divinyl concentration of 3 mg/m^3 had no effect on any of the test persons.

A comparison of thresholds determined for odor perception, eye sensitivity to light, and electrical brain activity (4, 3.8 and 3.6 mg/m^3, respectively) leads to the assumption that 3.0 mg/m^3 can be recommended as the maximum single-exposure concentration of divinyl in atmospheric air.

For 81 days (19 May to 7 August 1964) white rats were exposed continuously to divinyl to determine its resorptive action under condition of uninterrupted, prolonged inhalation, as well as to establish the average daily maximum tolerance dose. Four chambers with a volume of 0.1 m^3 (Fig. 4) were used. Air was run into the chambers at the rate of 30 l/min by a VPP-4 timed air blower. Divinyl in gaseous form was added to pure air.

Four groups of rats were used. Group 1 served as the control (pure air), and the remaining three were exposed to concentrations of 1.03 ± 0.006, 3.08 ± 0.006, and 30.8 ± 0.75 mg/m^3, respectively.

The divinyl concentrations in the chambers were checked daily by an SF-4 spectrophotometer in a gas cell, as well as after passing through a solid absorber. The temperature and humidity in the chambers were also checked during the inhalation exposure. These indices were comparatively stable. Animals were fed in the chambers twice daily and received a varied diet (boiled meat, milk, groats, bread, oats, grass, vegetables, and fish oil once a week). The state of the animals and changes occurring during the prolonged exposure to divinyl was checked by a variety of tests. Results are described in the text. Before initiating the test, the experimental animals were quarantined and observed for 2 weeks and background studies were conducted related to the indexes chosen as bases to evaluate the following changes.

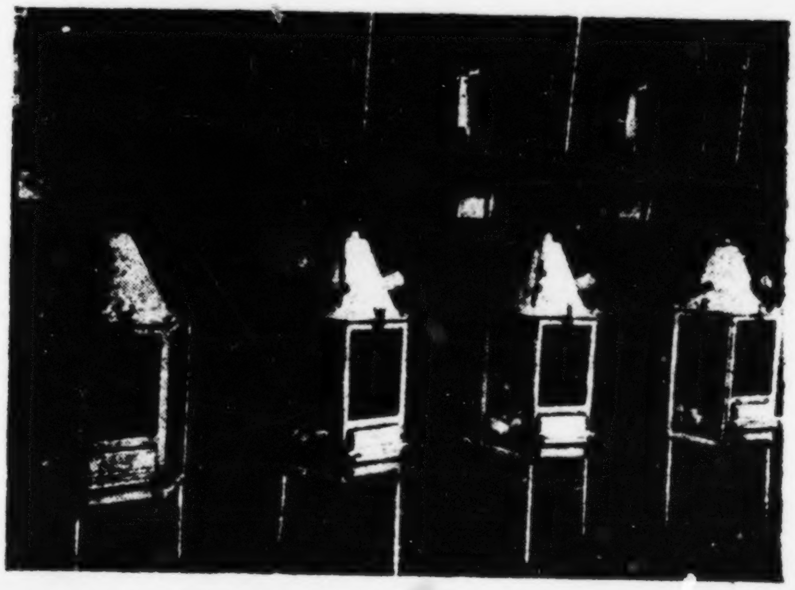

Fig. 4. General view of exposure chambers with dose controlling devices

Each chamber housed one group of 14-15 male rats of approximately the same weight. At the end of the inhalation exposure some animals were sacrificed immediately by decapitation, the living were kept under observation for an additional 2 weeks. At the end of the recuperation period these animals were also sacrificed and their organs studied histopathologically. The following anatomical and functional changes were noted in the course of inhalation exposure:

Animals' General Condition and Weight. During the preliminary inhalation days Group IV animals were restless probably due to the stimulating effects of the gas. This reaction was less noticeable in rats of Group III. Animals of the first three groups appeared healthy and active; they ate well throughout the exposure and no difference of any kind was noted in their general condition. They gained weight. However, at the beginning of the 3rd month rats of Group IV became visibly less active, their appetite fell as did their rate of gain in body weight (Fig. 5). The weight difference reached statistically reliable values only at the end of the exposure period. After the end of the inhalation exposure, Group IV rats gained weight rapidly.

State of Peripheral Blood. The blood of 5 rats in each group was examined every fortnight at a given time of the day. Blood was taken from the tail vein. The parameters investigated were erythrocyte count, hemoglobin (in a photoelectric erythrohemometer), and reticulocyte count. The red blood changes in the rats during the inhalation period were not pronounced.

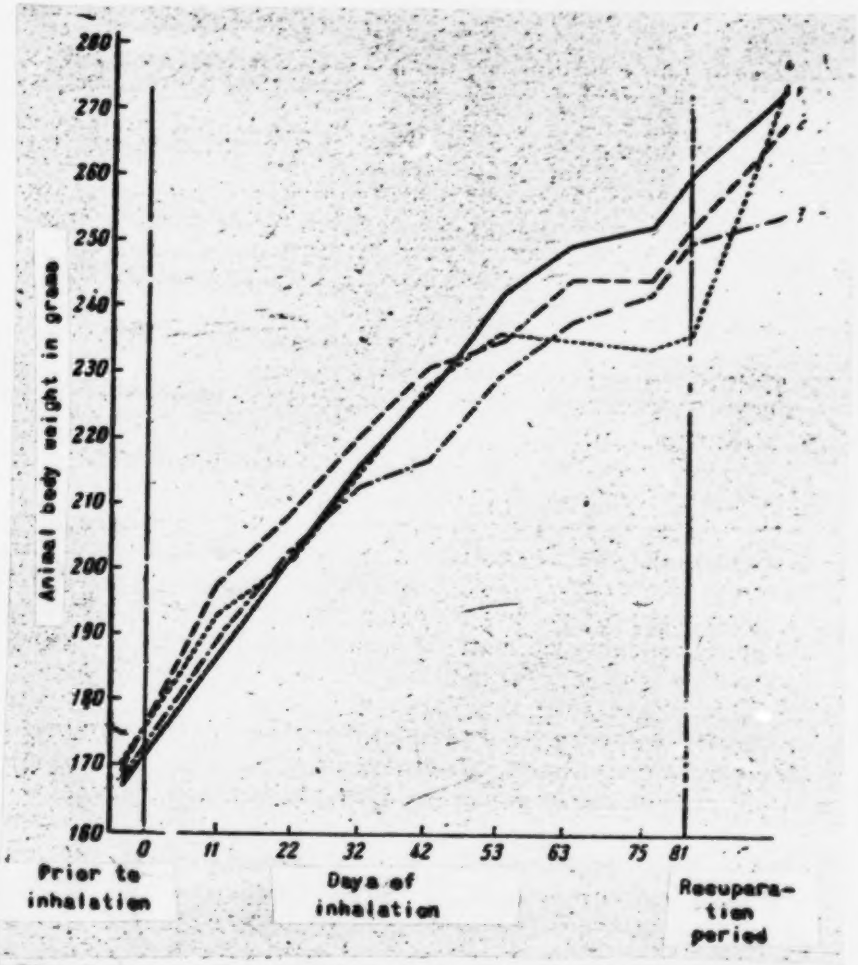

Fig. 5. Dynamics of average weight rate of different groups in the course of 24-hour divinyl inhalation.
1 - Control group; 2 - Group II; 1 mg/m^3 of divinyl; 3 - Group III, 3 mg/m^3; 4 - Group IV, 30 mg/m^3

Leucocyte counts were made by the generally accepted method including the leucocytic formula.

By the end of the inhalation period only rats of Group IV manifested a slight leucocytosis, which became statistically reliable only on the 80th day. Rats with slight leucocytosis also manifested shifts in the percent content of lymphocytes and stabnuclears, but these differences were not statistically reliable.

Activity of the Whole Blood Cholinesterase. Whole blood cholinesterase activity is related to the function of the mediators and unconditionally affected the state of the central nervous system and the transmittal of nerve pulses from the muscles. Whole blood cholinesterase activity also caused rapid hydrolysis of acetylcholine, and its inactivation allowed the latter substance to accumulate.

In the present study 5 rats from each group had been examined twice before inhalation exposure and once every fortnight during exposure by the Nicholls modification of the Limperos and Ranta method. This method is described by Merewether (1960). Visual determination of acetylcholine hydrolysis time served as the index of cholinesterase activity.

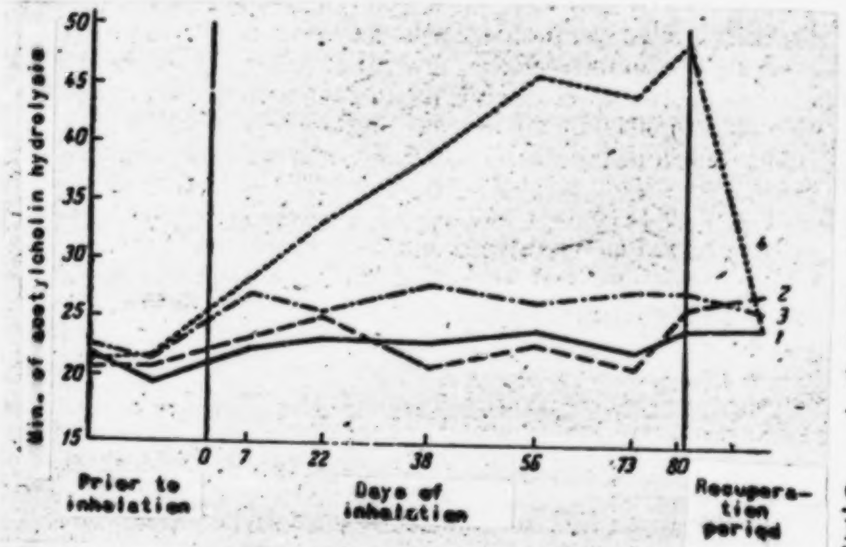

Fig. 6. Divinyl effect on activity of whole blood cholinesterase of different rat groups during continuous 24-hour continuous divinyl inhalation. Same notations as in Fig. 5.

As. Fig. 6 shows, changes in this activity were significant only in animals of Group IV. Statistically reliable differences were noted in this Group on the 7th, 38th, 56th, 73rd, and 81st days of exposure. Subsequently the indices returned to almost the initial values.

Optical Properties of the Blood Serum. The capacity of different substances to selectively absorb light in a given spectral band and at one or another wavelength is characteristic also of blood serum. Kamentskaya (1960) mentioned spectral data on the uv absorption by rabbit blood serum in the region of 230 to 300 millimicron wavelength, with maximum absorption at 275 and minimum at 250 millimicrons. This selective absorption is explained by the presence in rabbit serum of aromatic amino acids of tyrosince (maximum wavelength, 280 millimicrons) and tryptophane (maximum wavelength, 275 millimicrons). Kamentskaya believed that the changes in the absorption spectra of blood serum were related to the serum protein change, particularly tyrosine and tryptophane. Analogous data were reported by A. D. Semenko (1963), who studied the effects of low aniline concentrations on the optical properties of blood serum in rats. It has also been noted that the maximum and minimum serum absorption corresponded to 278 and 250 millimicrons, respectively.

The ratio of optical density at λ_{max} to that at λ_{min} fluctuated in healthy rats from 1.8 to 1.93 (absorption index).

Before being exposed to experimental inhalation 20 rats (5 from each group) had been examined to determine the spectral characteristics of their blood serum. Spectrophotometer SF-4 and quartz cuvettes with a layer thickness of 10 mm. were used. The serum was diluted 500 times by physiological solution. The maximum and minimum were identical with Kamentskaya's figures, but in the near uv (around 205-208 millimicrons) there was also considerable light absorption. This peak was not used in the present study because 205 millimicrons is actually beyond the resolving power of the SF-4. The serum-absorption index before inhalation exposure was 1.7-1.85. Even on the 9th day of exposure 3 rats of Group IV manifested a rise to 2.07-2.36, while on the 30th day the index for all rats in this Group stood at 2.03-2.36, where it remained to the end of the inhalation period, falling to a lower level during the recovery period. No marked shifts in this index were noticed in the rats of the other three animal groups.

Regardless of changes in the absorption index of Group IV animals, the location of the absorption bands did not change. Evidently shifts occurred, under the divinyl effects, in the content of aromatic amino acids, and these shifts altered the serum light absorption principally as a result of increased optical densities at λ_{max}=278 millimicrons. The general intoxication of the organism in connection as the result of divinyl inhalation by Group IV rats should have led to an increase in the content of several amino acids in the blood.*

The mechanism of a spectral shift clearly needs more detailed investigation, but the high sensitivity of the spectrophotometric method of studying optical properties of blood serum makes it possible to detect the finest changes in its protein composition. The index remained high for 2 weeks of the recovery period and did not return to the initial levels. Among rats of Groups II and III there were no significant shifts, through the inhalation period, either in absorption band or in optical density. Thus, the changes in optical blood serum properties observed in rats of Group IV point to changes in the state of serum proteins, associated with the effects of divinyl in 30 mg/m^3 concentration. At the same time, the absence of change in the other three groups indicated that divinyl concentrations of 1 and 3 mg/m^3 are inactive in this test situation.

Comparing the blood data brings out marked shifts in rats of Group IV with respect to leucocyte count, drop in cholinesterase activity, and changes in optical blood properties even in the absence of pronounced changes in red blood cells.

Arterial Pressure. The possibility of evaluating the effect of divinyl on the animal organism during continuous, prolonged inhalation exposure is related to the previously described hypotensive activity of divinyl under industrial conditions. A second monomer in the manufacture of synthetic rubber also possessed this hypotensive activity--alpha-methylstyrene (E. A. Kapkayev, 1963). The rats' arterial pressure was determined by an anemic method (V. M. Chernov, 1947; A. Kh. Kogan, 1959) in a caudal plethysmograph with a glass cuff attached to the caudal root. Pressure was recorded on a sphygmomanometer and oscillations on a water manometer. Arterial pressure was determined in 5 rats of each group after the animals were habituated to the experimental apparatus and the initial level of arterial pressure was ascertained. Subsequent arterial pressure checks were made during inhalation exposure once every 3 weeks during evening hours.

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*At the same time the effects of divinyl on the optical properties of the blood were being investigated, T. I. Lyutikovaya and the present author conducted a similar study in which white rats were inoculated with cracking-process gas. Here, too, there were marked shifts in the absorption index.

Arterial pressure was stable in all rat groups before inhalation exposure, and for more than 2 months of the experimental exposure (Fig. 7). However, on the 72nd inhalation day the average pressure in Group IV dropped from 90-93 mm Hg to 75-77; it remained at this level to the end of the inhalation test and throughout the recovery period. Beginning with the 72nd day this blood pressure drop was statistically reliable. No change was noted in the arterial pressure of the other rat groups. Thus, according to this test the effective divinyl concentration was 30/mg/m^3.

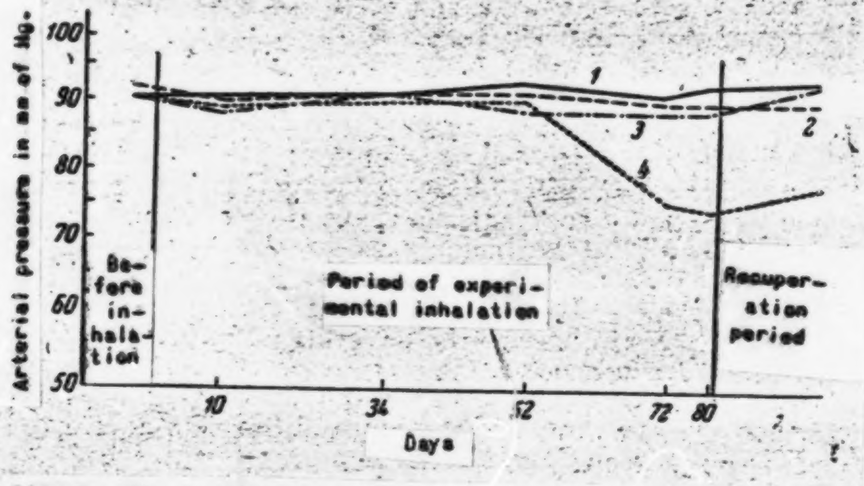

Fig. 7. Blood pressure changes in rats of different groups in the course of continuous 24-hour divinyl inhalation. Same notations as in Fig. 5.

Animals' Overall Motor Activity. Evaluation of the divinyl effects on the central nervous system and the prevailing stimulation or inhibition in the cerebral cortex was made by the method of objective recording of total motor activity based on the principles of actograph. The actographic method has been employed in recording motor and conditioned reflex activity and in studying the daily periodicity of the animal organism (A. D. Slonim, 1953; N. I. Kalabukhov, 1940; Ya. B. Maksimovich, 1954; O. S. Kishakovskiy, 1954). Impulses were recorded by a kymograph connected with pneumatic capsules, cushions, electromechanical counters, etc. Special organic glass chambers were built with a sectioned bottom consisting of seven movable plates fastened in the center on semiaxes as shown in Fig. 8. When the pressure on one half-plate exceeded 0.5 g/m$_2$, an electric contact under the plate closed, making it possible to record every movement of the animal in the chamber. The contacts were connected to an electromagnetic mechanical counter in a 12v d.c. circuit. During the study the counter recorded all incident pulses.

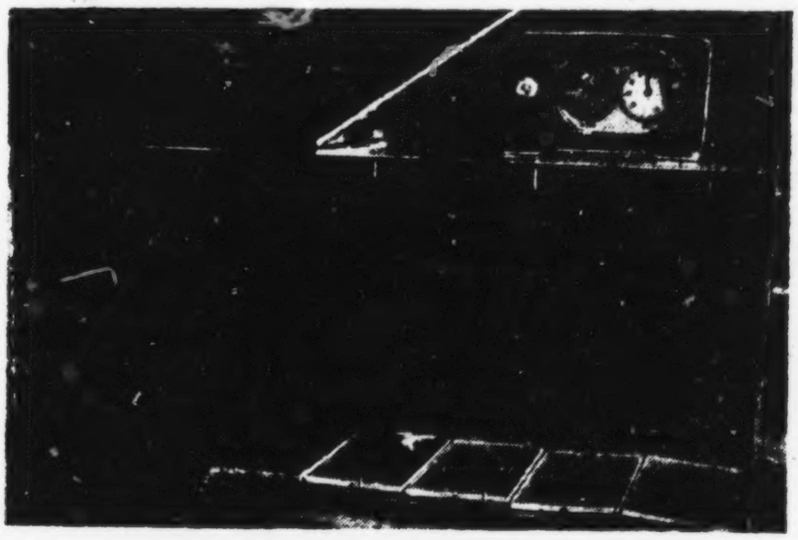

Fig. 8. Chamber for the study of total motor activity of experimental animals.

Three such chambers were used, each of which housed a rat for 3 hours. Each rat was examined not less than 3 times weekly. Five rats of each group were studied actographically. Before inhalation exposure, rats were oriented to the equipment in the chambers over a period of several days. On the 2nd or 3rd day the rats became adapted to the equipment, and their behavior in the chamber became normalized. Only in the first 5-10 min. after being placed in the chambers did the rats evidence any reaction to the environmental change. In order to exclude even this, the counter was introduced 15 min. after the animal was placed into the chamber. The background of total motor activity was studied for 3-4 days, the average data during the initial period was taken as 100 percent. Later, motor activity was totaled by weekly inhalation periods for each animal group. Resulting data are presented in block form in Fig. 9. The degree of change (reduction) in motor activity in rats of Group IV beginning on the 7th week of inhalation exposure somewhat exceeded the changes in weight, general well being and arterial pressure of the rats. The objective-recording method made it possible to record these shifts earlier than they appeared in the previous investigations. The fall in total motor activity affected rats of Group IV to the end of the inhalation exposure and through the recovery period.

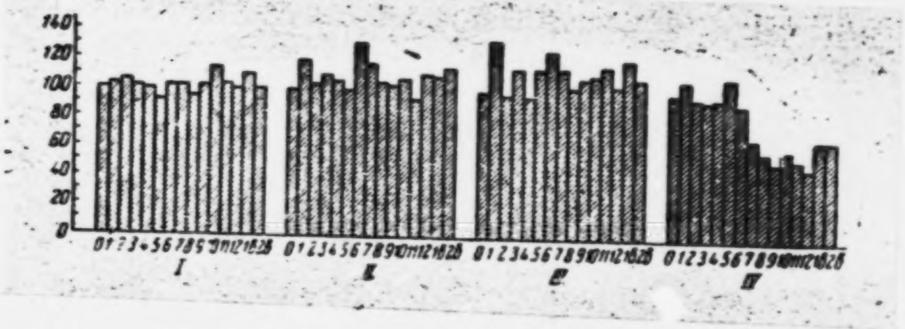

Fig. 9. Changes in motor activity of rats during continuous 24-hour exposure to divinyl inhalation, evaluated on the basis of weekly periods and in percent of initial values.
I-IV animal groups; 0 - basic background; 1 - 12 exposure weeks; 1b - 2b weeks of recovery period.

The differences in Group IV (compared with the controls) are statistically reliable. Rats of Groups II and II manifested no significant changes as compared with the control rats. According to this test, divinyl was effective only in a concentration of 30 mg/m^3.

Results of Pathohistological Examination.* Sections of the liver, kidneys, heart, spleen, brain, and bone marrow (femur) were used. In addition, the mucous membrane of the tip and pharynx of the nose were examined.

The specimens were embedded by the usual methods, sectioned and stained with hematoxiline-eosine. Brain specimens were also embedded in celloidine, sectioned and stained by the Nissl method. Results were as follows: the nasal mucous membrane was not intact even in animals of the control group, which manifested focal and in isolated instances, diffuse infiltration; the epithelium appeared normal. The recorded changes may have been caused by factors in the external medium (mechanical effects in connection with the large number of animals in the chamber, air humidity, etc.). Group III animals (3 mg/m^3 manifested disintegration of the mucous-membrane epithelemium more frequently than the control group. A greater difference was manifested by rats of Group IV (30 mg/m^3), which showed pronounced inflammatory infiltration of the mucous membrane. In 3 animals the process was diffuse, with massive exudation from the nasal cavity.

The lungs of Group II rats did not differ from those of the controls. Most rats of Group III exhibited a moderate intermediate pneumonia and small atelectasis foci and emphysema against a background of well-developed lymphoidal tissue. There were small atelectasis foci and emphysema also well-developed hyperplasial lymphoidal tissue in nearly all rats of Group IV. Many of the animals exhibited obvious intermediate infiltration, perivascular edema, and interstitial pneumonia. No histopathological changes were noted in the brain sections of any control or experimental rats. The same was true of the bone marrow. Only animals in Group IV manifested hemosiderin accumulation in the red pulp of the spleen.

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*V. V. Veselovaya investigated and described the microscopic specimens.

Many animals of Group IV exhibited thinning of the hepatic columns with almost total absence of clear cells, which predominated in the liver of the other three groups. It must be assumed that these cells were rich in glycogen. Individual rats of Group IV showed edema of the endothelium of the sinuses. Marked changes were noted in the kidneys of Group IV rats. Similar, but less pronounced, processes were seen in rats of Group III, appearing as swelling of the epithelium of the convoluted tubules and the appearance of protein and individual hyaline cylinders in the tubule lumens.

Data obtained from all tests during rats' inhalation exposure, pathological investigation excepted, divinyl in a concentration of 1 or 3 mg/m^3 caused no noticeable changes in the general state of the animals, significant results being obtained in most tests only at 30 mg/m^3 of divinyl. Morphological changes induced in animals exposed to the inhalation of divinyl in concentraion of 3 mg/m^3 indicate that the gas has a stimulating effect on the mucous membranes of the respiratory tract, on the lungs, and on the kidneys. A concentration of 1 mg/m^3 was ineffective, and can be recommended as the average daily concentration in atmospheric air.

In 1964 this author investigated divinyl as an atmospheric air pollutant in the vicinity of a synthetic rubber plant which began to operate in 1963, producing divinyl--alpha-methylstyrene rubber. At the present time these two basic monomers are produced locally. Divinyl is produced by dehydration of butane released by an oil refinery. A rosin emulsifier is used for polymerization. The following are potent sources of divinyl discharged into the atmosphere: polymerization and monomer-distillation shops, shops where divinyl is extracted from a copper-ammonia complex by chemiabsorption, and the divinyl storage shop.

The investigation was conducted in the July-August period (170 samples) and November-December of 1964 (150 samples) at outside-air temperatures of plus 25 to 30° C in the summer and minus 7 to 15° in the winter. The samples were passed through a solid sorbent in U-shaped absorbers with aqueous aspirators or pumps from PkhR-54-MV equipment. During summer months the divinyl concentration exceeded the proposed maximum single-exposure tolerance level (3 mg/m^3) at only three points: 50 and 70 m from the divinyl extraction shop (6.12 and 6 mg/m^3) and 30 m from the monomer distillation shop (3.31 mg/m^3).

During the winter months excessive amounts were discovered only at 20 m from the same shops (3.2 and 3.5 mg/m^3). At 500 m the divinyl concentration was 0.2-0.7 mg/m^3, while at a radius of 1500-2000 m there were no traces.

The low boiling temperature of divinyl (minus 4° C) and the high temperature of the ambient air during the summer (20-30°) caused considerable escape of the gas from the shops. During the winter months divinyl concentration was lower at all sampling stations. In this connection special attention should be focused on making all systems in the shops absolutely leak-proof, particularly during the summer, to reduce the discharge of divinyl into atmospheric air.

CONCLUSIONS

1. On the basis of the present study it is recommended that 3 mg/m^3 be adopted as the maximum single-exposure concentration in atmospheric air.

2. On the basis of results obtained in this study under conditions of prolonged, chronic divinyl inhalation, it is suggested that 1 mg/m^3 be adopted as the permissible average daily concentration of divinyl in atmospheric air.

3. To determine microquantities of divinyl in atmospheric air in the presence of other products of synthetic rubber production , it is suggested that a spectrophotometric method be employed which retains the divinyl on a solid sorbent with subsequent spectrophotometric determination of the elated isooctane solution in an SF-4 spectrophotometer.

TOXICITY OF ETHYLENE OXIDE IN LOW CONCENTRATIONS

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Ethylene oxide (oxirane) was discovered by A. Wuertz in 1857; it is a colorless gas at room temperature having the odor of ether and a caustic taste. At low temperatures it turns into a fluid which is more volatile than either. Its boiling and freezing points are, respectively, 10.7 and -111.7°C. In a 3 to 80 percent mixture with air it can be explosive. It is soluble in water, alcohol, ether, and other material containing free hydrogen atoms. Ethylene oxide (EO) easily interacts with many organic and inorganic substances and is stable at high temperatures. There are two ways to produce EO: by the interaction of alkalies with ethylene chlorohydrin, and by direct catalytic oxidation. EO is used in the organic-synthesis industry to produce glycol and polyglycol esters, acrylonitrile, and acrylic acid esters and their polymers, used in the manufacture of organic glass, synthetic fibers ("orlon"), and synthetic rubber. In addition EO is used in the production of textiles, lubricants, and plastic solvents; also as a pesticide, in daily life, and as a disinfectant in medicine. EO is a highly poisonous narcotic, which may be due to the formation in the organism, as a result of EO, of formaldehyde or ethylene glycol with subsequent formation of oxalic acid. It is also possible that EO combined with trimethylamine to form an active acetylcholine, or it may react with the amino groups of proteins (N. V. Lazarev, 1963). According to Lazarev a 10 min. exposure to an atmosphere containing EO vapor will produce severe vomiting, dizziness, sweet taste in the mouth, and - for several weeks thereafter - impairment of cardiac activity. E. M. Bongard and V. F. Shlyapin (1958) examined the health of workers coming in direct contact with EO. Acute EO poisoning occurred in workers under emergency conditions, when the EO concentration became high. Acute EO poisoning affected the nervous system and normal psychological functions. Soviet and foreign studies agreed regarding the toxicity of relatively high EO concentrations; however, no biological effects of small concentrations in atmospheric air were recorded in the literature. Using the M. V. Alekseeva modification of the E. Sh. Gronsberg method, the author conducted a study - first, for the determination of the odor perception threshold - of EO in atmospheric air.

Data in Table 1 show that the odor perception threshold concentration in the most sensitive subjects was 1.5 mg/m^3, and the maximum odor nonperceptible, 1.0 mg/m³.

Table 1

RESULTS OF ETHYLENE OXIDE THRESHOLD ODOR DETERMINATIONS

No. of observations	Min. percep. concn. in mg/m³	Max. nonperceptible concns. in mg/m³	Total observations
2	1,5	1,0	75
3	1,9	1,5	103
3	2,7	1,9	99
2	3,5	2,7	71
2	4,3	3,5	53
6	5,4	4,3	170
2	More than 6.6	—	40

Studies on EO effect on eye sensitivity to light showed that for the most sensitive persons the minimum odor perception threshold was 1 mg/m³, while the concentration of odor perception subthreshold was 0.5 mg/m³ (Table 2, Fig. 1).

The effect of EO on the electrical activity of the cerebral cortex in man was studied using an 8 - lead Kaiser EEG equipped with engineer B. N. Balashev's integrator; the A. D. Semenko method was followed, by which the subject's EEG was recorded, while a rhythmically pulsing light of optimum frequency and variable intensity (0.1-0.6 j) was used as the stimulation. The EEG helps to determine the nature of amplication of the alpha-rhythm's natural potential.

Table 2.

EYE SENSITIVITY TO LIGHT CHANGES DURING EXPERIMENTAL ETHYLENE OXIDE INHALATION

Test persons	Odor percept. threshold in mg/m³	% of fresh air, M	2.4 мг/м³			1.0 мг/м³			0.5 мг/м³		
			M (%)	t	Reliability degree	M (%)	t	Reliability degree	M (%)	t	Reliability degree
Z.Kh	1,5	205	642	5,5	c	312	2,5	a	178	0,95	o
E.B.	1,5	327	427	2,9	a	445	3,6	b	389	1,1	o
L.K.	2,7	159	166	0,39	o	133	1,9	o		—	—

Note: Reliability: a - 95%; b - 99%; c - 99.9%

After several tests with pure air, the subject, unknown to him, was given inhalation of EO for 3 min. in a concentration below the odor perception threshold; the effect of this concentration is judged by changes elicited in the alpha-rhythm. Tests were made with EO in concentrations of 2. 3, 1.4, 0.65, and 0.3 mg/m³. For the group of volunteers the threshold of odor perception was 1.9-2.7 mg/m³, while 0.65 was the minimum concentration which elicited changes in alpha-rhythm potential. A concentration of 0.3 mg/m³ was inactive (Fig. 2); it is here proposed as the maximum single-exposure concentration in atmospheric air (Table 3).

Maximal allowable average daily concentrations were determined by means of chronic experiments using 60 white male rats weighing 95-130 g. Rats were divided into 4 groups of 15, three of which were exposed to continuous 83 days inhalation of the following EO concentrations in mg/m³:

I, 0.3 (the above recommended single-exposure tolerance); II, 0.03, III, 0.01; and IV, control. The EO concentration in each chamber was checked daily. The range of averages for Groups I, II, and III, respectively, was 0.31 ± 0.0069, 0.03 ± 0.0016, and 0.015 ± 0.0069 mg/m³. The parameters used in judging EO effects were: general behavior and body weight gain, effect on chronaxy ratio of the antagonist muscles, change in whole blood chlorides and residual nitrogen content, changes in blood serum protein fraction picture, and the results of pathomorphological and pathohistological examination at the end of inhalation and the recovery periods.

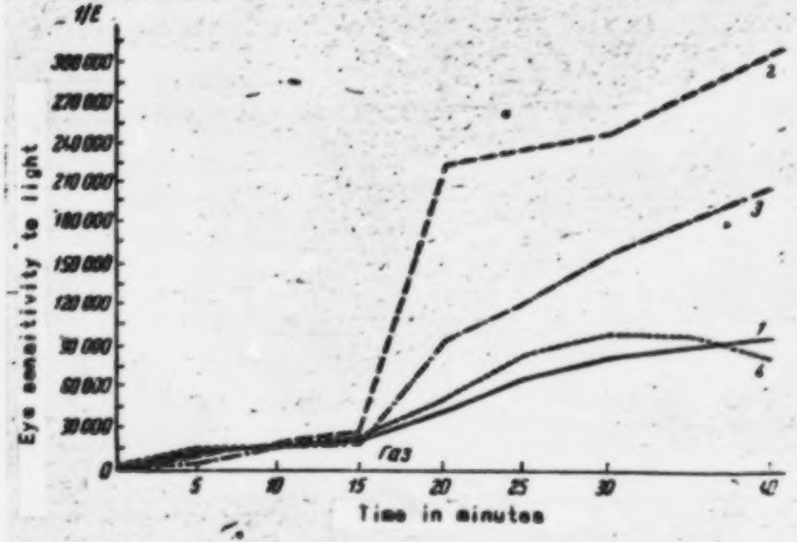

Fig. 1. Ethylene oxide effect on eye sensitivity to light of female Z.A.Kh.
1 – Clean air; 2 – 2.4 mg/m³; 3 – 1 mg/m³; 4 – 0.5 mg/m³

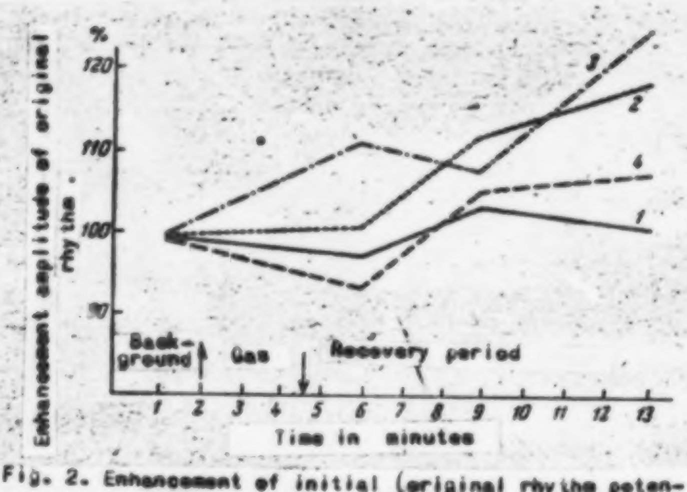

Fig. 2. Enhancement of initial (original rhythm potentials during ethylene oxide inhalation by female R.I.
1 – Clean air; 2 – 1.4 mg/m³; 3 – 0.65 mg/m³; 4 – 0.3 mg/m³

Table 3

SUMMARY OF TABLE OF RESULTS OBTAINED BY STUDY OF ETHYLENE OXIDE EFFECT ON MAN'S REFLEX ACTIVITY

Reaction studied.	Mg/m^3 of ethylene oxide	
	Threshold concn.	Sub-threshold concn.
Oder perception	1,5	1,0
Eye sensitivity to light	1,0	0,5
Electrical activity of brain cortex	0,65	0,3

Animals of all groups were active throughout the inhalation period and no statistically reliable changes in weight were found. Five rats of each group were examined every 10 days, under identical conditions and at the same hour of the day, and to check on the hind leg muscles chronaxy ratio; a response was recorded on an ISE-01 pulse chronaxymeter when the animal jerked its foot. The muscle-antagonist chronaxy ratio constitutes a sensitive test which reflected functional shifts in the central nervous system when low concentrations of toxic substances were inhaled (K. A. Bushtuyeva, 1961; V. A. Gofmekler, 1960; Yu. G. Fel'dman, 1962; R. Ubaydullayev, 1961; V. A. Chizhikov, 1963; and others).

Rats of Group I (0.3 mg/m^3) experienced an inverse antagonist ratio on the 8th week of exposure to inhalation (increase in flexor chronaxy) which persisted to the end of the experimental inhalation period. These changes reverted to normal during the recovery period. The statistical reliability of the changes was 0.95-0.999. No reliable changes were observed in the antagonist ratio in rats of the other three groups (Fig. 3).

On the basis of data presented by P. V. Zimakov (1946) and N. V. Lazarev (1963) indicating that EO affected the kidney function and the mineral salts content in the blood, which is a reliable index of the harmful activity of several unfavorable factors, a study was initiated to determine the dynamics of chlorides and residual nitrogen in the whole blood of white rats. Chlorides were determined in 5 rats of each group once every 15 days, using the Levinson method based on the fact that chlorides yielded insoluble silver chloride with silver nitrate. The proteinless filtrate of the blood, containing the chlorides, is titrated by a solution of silver nitrate in the presence of calcium chromate to a change of color.

In rats of Group I the change in blood chloride content appeared at the end of the second month of inhalation exposure; it took the form of a reduction in chloride concentration from 458.3 mg percent of 375.6 (controls: 469.5). Statistical reliability of these changes was 0.999. The content of chlorides returned to normal (Fig. 4) during the recovery period.

No reliable change in the blood chloride content was noted in rats of Groups II, III, and IV. After 20 days the residual blood nitrogen was determined in 5 animals of each group; Convee cups were used in the chloride determination, a method based on the fact that organic matter decomposed by sulfuric acid in the presence of a catalyzer formed amonium sulfate.

Ammonia was set free by the addition of an alkali. Sulfuric acid which did not react with the ammonia, is titrated with an alkali. The amount of bound acid corresponded to the amount of ammonia set free, which in turn corresponded to the amount of nitrogen.

A change in the residual-nitroben content of the blood appeared in the Group I rats during the latter half of the second month of inhalation exposure rising from the initial level of 36.4 mg% to 56.3 and then returning to normal level during the recovery period. The control reading was 38.1. The reliability of this change was 0.95.

Rats of Groups II and III exhibited no reliable change (Fig. 5). Paper electrophoresis tests were made every 20 days to determine the effects of EO on the protein fraction picture of the blood serum using 5 rats of each group: throughout the inhalation period no reliable changes in the overall amount of protein and protein fractions were observed. In summarizing the results, it

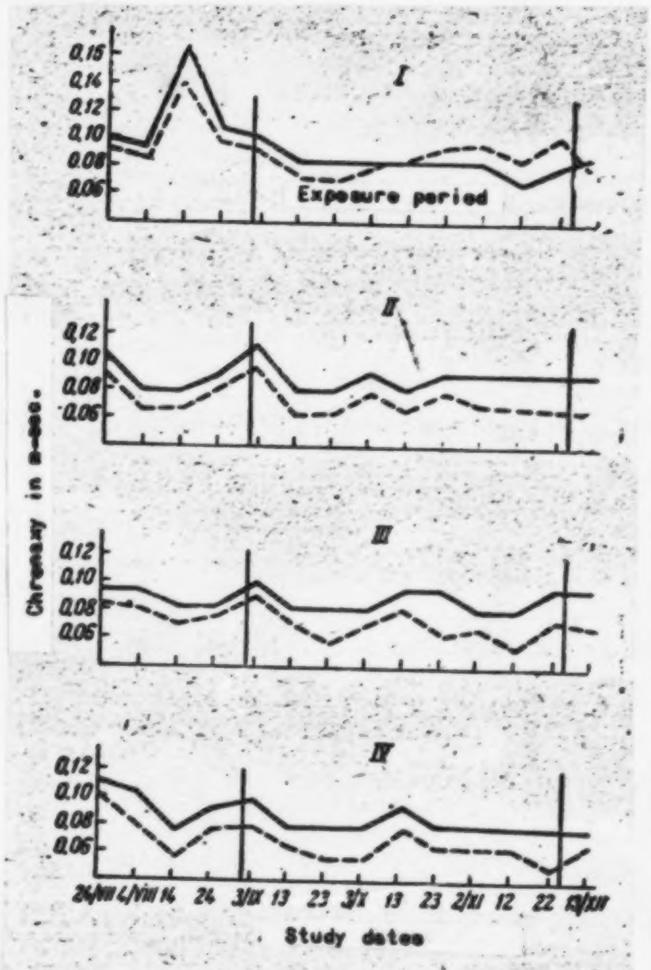

Fig. 3. Muscle antagonist chronaxy ratio in experimental animals affected by ethylene oxide. I, II, III, and IV - groups studied; solid curves - extensors; broken line curves - flexors.

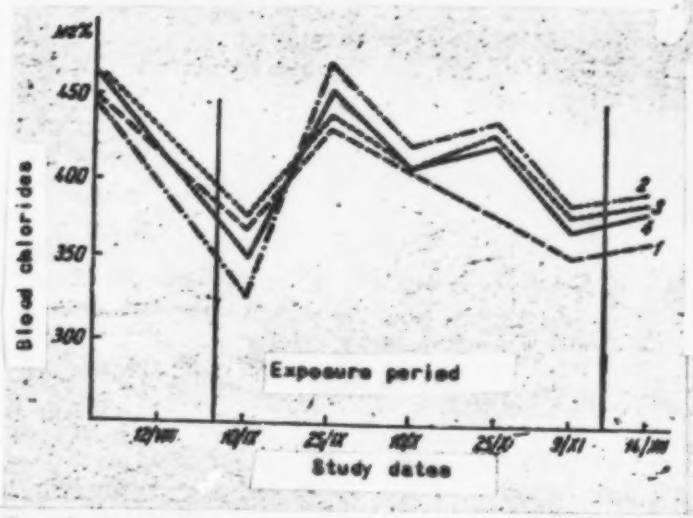

Fig. 4. Ethylene oxide effect on whole blood chlorides in rats of different groups
1 - 0.3 mg/m^3; 2 - 0.03 mg/m^3; 3 - 0.01 mg/m^3; 4 - control

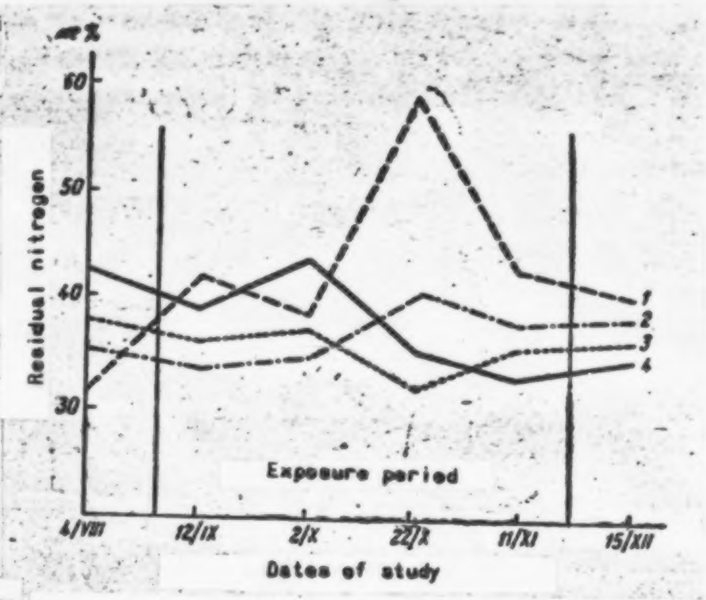

Fig. 5. Effect of ethylene oxide on residuan nitrogen in whole blood of rats of different groups. Notations same as in Fig. 4.

can be stated that chronic 83-day exposure to EO inhalation of white rats in a concentration of 0.3 mg/m^3 elicited pronounced changes in the antagonist muscles chronaxy ratio and in the concentration of chlorides and residual nitrogen in the blood. An EO concentration of 0.03 mg/m^3 elicited no changes in any of the tests. Therefore, this concentration is recommended as the average daily maximal concentration in atmospheric air.

To determine the extent, type, and intensity of air pollution, a survey was conducted in the summer of 1967 in the proximity of the EO manufacturing plants: single-exposure air samples were collected at 50 to 500 m from a leeward discharge source (Table 4).

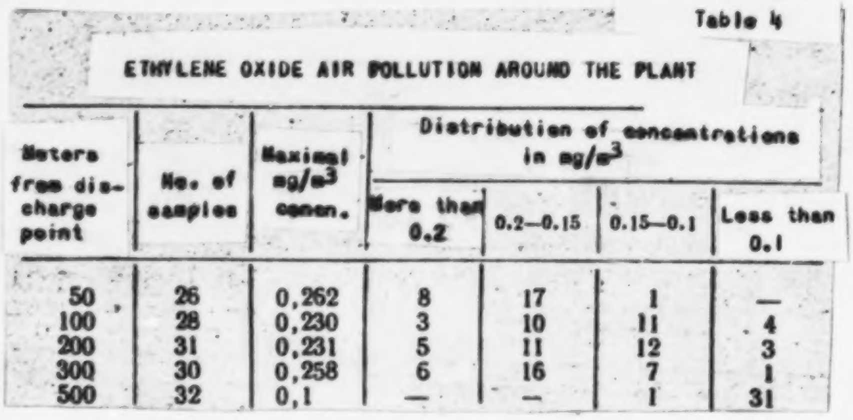

Table 4

ETHYLENE OXIDE AIR POLLUTION AROUND THE PLANT

Meters from discharge point	No. of samples	Maximal mg/m^3 concn.	Distribution of concentrations in mg/m^3			
			More than 0.2	0.2—0.15	0.15—0.1	Less than 0.1
50	26	0,262	8	17	1	—
100	28	0,230	3	10	11	4
200	31	0,231	5	11	12	3
300	30	0,258	6	16	7	1
500	32	0,1	—	—	1	31

Results of the study showed that EO air pollution around the plant in an area of 500 m radius, the EO concentrations were substantially below those recommended for a single-maximal exposure. Serious consideration should be given the probability that if plant production were to be enhanced by the introduction of new technology (e.g., EO production by direct oxidation), the intensity of atmospheric polution might considerably increase. In this connection it must be borne in mind that in establishing boundaries for the sanitary protection zone, the presence in the proximity of other plants discharging air pollutants should be taken into account.

CONCLUSIONS

1. Ethylene oxide as a poison affected all body organs and systems, principally the central nervous system.

2. The EO odor perception threshold for most sensitive test persons was found to be 1.5 mg/m^3; threshold eye sensitivity to light 1.0 mg/m^3; and threshold of electrical cerebral cortex activity 0.65 mg/m^3.

3. It is recommended that 0.3 mg/m^3 of EO be adopted as the limit of its concentration in atmospheric air.

4. Chronic, 24-hr. exposure of white rats to the inhalation of air containing 0.3 mg/m^3 of EO for 83 days produced changes, affected the animals' flexor-extensor chronaxy ratio and the chloride and residual nitrogen blood contents.

5. It is suggested that the average daily maximal allowable EO concentration in atmospheric air be set at 0.03 mg/m^3.

6. Atmospheric air pollution with EO has been demonstrated as far as 500 m from the discharge source.

A STUDY OF HYGIENIC PROPERTIES OF METHANOL AS AN ATMOSPHERIC AIR POLLUTANT

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Methanol is produced synthetically and by hydrolysis in large quantities. It is used widely in the manufacture of organic dyes, pharmaceutical compounds, formaldehyde, and other chemicals. The main sources of methanol as an air pollutant are plants manufacturing paint, varnish, wood-chemistry products, furniture making and some metal working plants. Methanol is a nerve and blood vessels poison the effects of which are cumulative. L. I. Kazas (1925) and V. M. Rozhkova (1948) consider that the basic poison effects of methanol came from its primary oxidation products in the organism, viz., formaldehyde, which arrested cell respiration and inhibited the oxidation functions of the body. The effects of low methanol concentrations on the living organism have been studied by Chiao Chen-Tsi (1959), who experimentally determined the concentration of its odor perception threshold (4.1 mg/m^3) and threshold of reflex on eye sensitivity to light (3.3 mg/m^3); he also conducted chronic inhalation series with white rats and methanol vapor in concentrations of 50 and 177 mg/m^3 for 12-hr. daily intervals for 3 months. These studies served as a basis for setting the maximum allowable single-exposure and maximal average-daily concentration limits in atmospheric air at 1.5 and 0.5 mg/m^3, respectively.

However, in setting the single-exposure concentration level the authors did not employ the most sensitive index, which is electrical cerebral cortex reflex activity and continuous 24 hr. inhalation exposure. In the present paper error by omission was corrected by using the most modern experimental procedures. Methanol concentration in the atmospheric air was determined by the method of M. V. Alekseyeva (1963). First, the threshold of methanol odor perception was determined in 25 test volunteers 18 to 40 years old. Nine methanol concentrations ranging from 3.9 to 12.2 mg/m^3 were used, and results reported in Table 1.

Table I

DETERMINED THRESNOLDS OF METHANOL VAPOR ODOR PERCEPTION

Nos. of test Persons	Minimal perceptible concn. in mg/m^3	Maximal nonperceptible concn. in mg/m^3
3	10,3	9,7
5	8,4	7,5
4	7,5	6,5
2	6,5	5,6
5	5,6	4,5
6	4,5	3,9

Data in the last line show that the minimum perceptible and maximum nonperceptible concentrations were 4.5 and 3.9 mg/m^3, respectively, closely approximating the 4.1 mg/m^3 recommended by Chiao Chen-Tsi. The lowest methanol concentration which upon inhalation affected eye sensitivity to light was studied next, using an ADM adaptometer and three test persons aged 18 to 25. Tests were made once daily on each individual under identical conditions and at a standardized time of day.

The physiological background - i.e., the normal curve of eye adaptation to the dark or sensitivity to light - was determined on 8 successive days by 15- and 20-min. pure air inhalations. A 4.11 mg/m^3 methanol concentration elicited a sharp change in the light subjects' eye sensitivity to light, while only one individual reacted to a concentration of 3.52 mg/m^3; the inactive methanol concentration for this individual was 3.06 mg/m^3 (Fig. 1).

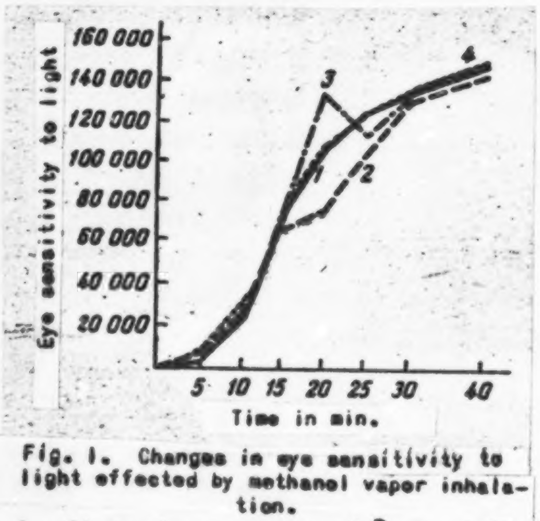

Fig. 1. Changes in eye sensitivity to light effected by methanol vapor inhalation.
1 - Clean air; 2 - 4.11 mg/m^3; 3 - 3.53 mg/m^3; 4 - 3.06 mg/m^3.

Effect of low methanol concentrations on the electrical cerebral cortex reflex activity was investigated by the method of A. D. Semenenko (1963) using an 8-lead EEG. Six subjects most sensitive to olfactory stimuli were selected for this test (Table 2). At least 4 tests were made with each concentration, and the methanol inhalation was alternated by tests with pure air.

Results of this investigation showed that methanol in 1.46 mg/m^3 concentration affected the alpha-rhythm amplitude; however, this was true in only 2 cases of the group which inhaled air containing methanol in concentration of 1.17 mg/m³. The 1.01 mg/m³ concentration proved inactive in all test persons. Results are shown in Table 3 indicating that the maximal methanol concentration of single inhalation determined in earlier studies (1.5 mg/m³) needs to be lowered to 1 mg/m³.

Table 2

METHANOL VAPOR REFLEX EFFECT ON ELECTRICAL BRAIN CORTEX ACTIVITY

Test persons	Mg/m³ of methanol		
	1.46	1.17	1.01
G. K.	+	+	—
P. K.	+	—	—
T. A.	+	Not investigated	—
M. K.	+	—	—
L. T.	+	+	—
L. M.	+	Not investigated	—

Remark: + statistically reliable and - statistically non-reliable changes

Table 3

METHANOL THRESHOLD ODOR AND REFLEX EFFECT ON THE HUMAN ORGANISM

Threshold	According to Chao Chen-Lee		According to this author	
	Concentrations in mg/m³			
	Minimal active	Maximal inactive	Minimal active	Maximal inactive
Odor perception	4,1	3,7	4,5	3,9
Eye sensitivity to light	3,3	2,4	3,53	3,06
Electrical activity of brain cortex	Not studied		1,17	1,01

In order to check the average-daily exposure limits 45 white male rats were subjected to chronic, 24-hr., 90 day inhalation exposures. The rats, which weighed 100 to 200 g, were divided into three groups of 15 specimens; they were tested by following methanol concentrations, in mg/m³: Group I, 5 (somewhat higher than the odor perception threshold); Group II, 0.5 (the existing average daily maximum allowable concentration); and Group III, controls. The air temperature and methanol concentrations had been checked in each chamber daily in the course of the study. Average methanol concentration for Group I was 5.31 ± 0.62 mg/m³, for Group II, 0.57 ± .059 mg/m³. During the inhalation period animals of all groups were healthy, active, and gained in body weight. However, toward the end of the experimental period the control animals lost some neglible amount of weight.

Once every 10 days 5 rats of each group were checked for their motor chronaxy ratio. Prolonged inhalation of methanol vapors by rats in Group I (5.3 mg/m³) manifested statistically reliable changes beginning with the 6th week, also a paradoxical and inverse ratios of antagonist chronaxy. At the end of the recovery period the chronaxy ratio of flexor and extensor muscles returned to normal.

Rats of Group II manifested no changes in their chronaxy indices (Fig. 2).

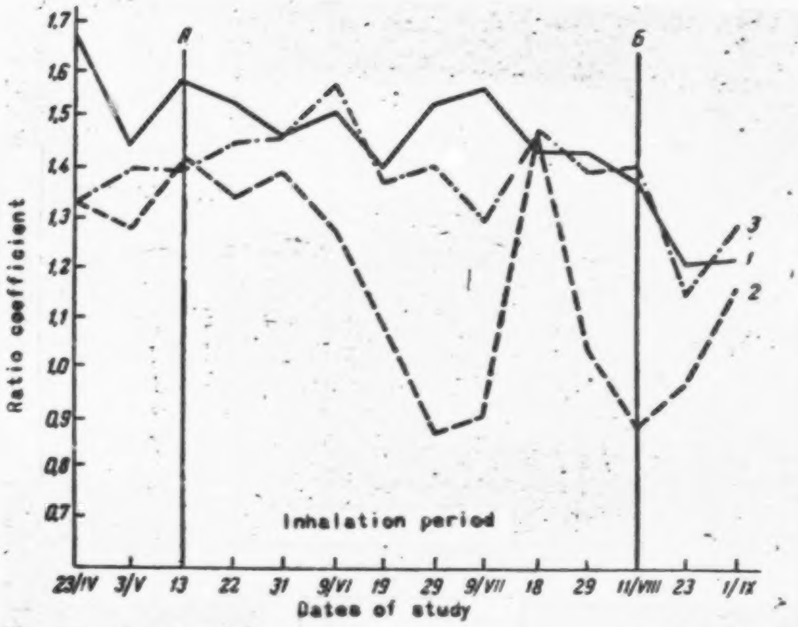

Fig. 2. Muscle antagonists chronaxy ratio changes in rats effected by the inhalation of methanol vapor.
A and B period of inhalation. 1 - Clean air; 2 - 5.31 mg/m^3; 3 - 0.57 mg/m^3

M. I. Gusev (1960), K. A. Bushtuyeva (1964), B. M. Mukhitov (1961) and others have noted changes in the excretion of coproporphyrin with the urine of the animals during prolonged action of several harmful chemical agents, a physical phenomenon which was carefully studied here. Daily urine samples had been collected from 5 rats of each group into specially made glass containers. Porphyrin was extracted from the urine by the Fisher method; the amount of coproporphyrin was determined once every two weeks with an SF-4 spectrophotometer in the 400-410 millimicrons range. The amount of coproporphyrin excreted via the urine of Group I rats per 100 g of body weight dropped sharply beginning with the 7th week and then stayed at this level for the duration of the inhalation period (Fig. 3). Normalization set in on the 20th day of the recovery period. No statistically reliable changes were observed in rats of Group II.

Whole blood cholinesterase activity was determined, colorimetrically by the A. A. Pokrovskiy (1953) method as modified by A. P. Martynova (1957), using 5 rats of each group fortnightly. Before experimental inhalation acetylcholine hydrolysis time averaged 38-39 min. Beginning with the 6th week, hydrolysis time for rats of Group 1 rose to 41 min., indicating a decrease in cholinesterase activity. By the end of the inhalation period the time had risen to 43 min. (Fig. 4), after which it returned to normal. No reliable changes were found in rats of Group II.

Reports found in the literature clearly indicate that total blood protein and the blood protein fractions picture were affected by disease and by different chemical substances (R. Ubaydullayev, 1961; V. A. Chizhikov, 1964; P. G. Tkachev, 1964; Granati, Sekavo, 1956; Mario, Carlo, 1957. Therefore, the action of low methanol concentrations on protein fractions of blood serum was studied experimentally by the method of paper elec0 trophoresis in 5 rats of each group. Blood was taken from the tail vein on an empty stomach every 15 days.

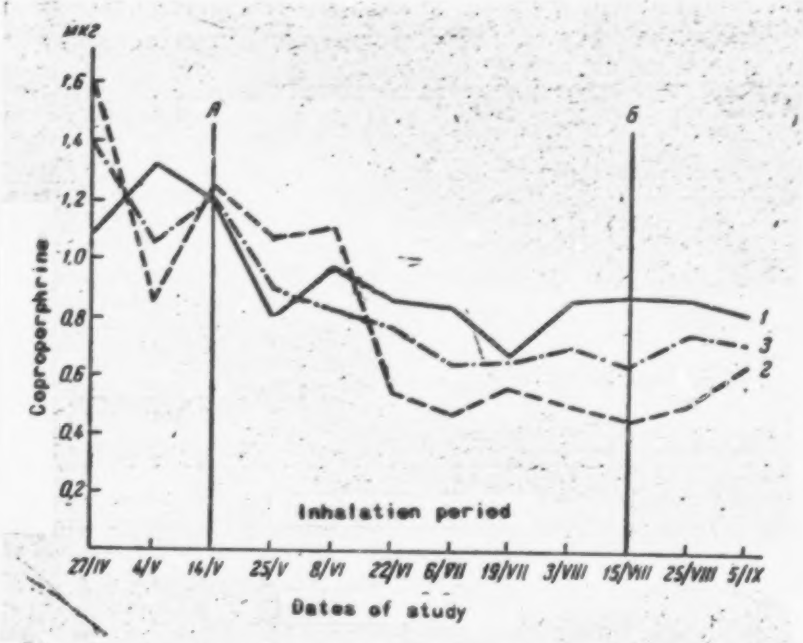

Fig. 3. Copreporphrine elimination via the urine by rats during methanol vapor inhalation. Notations same as in Fig. 2.

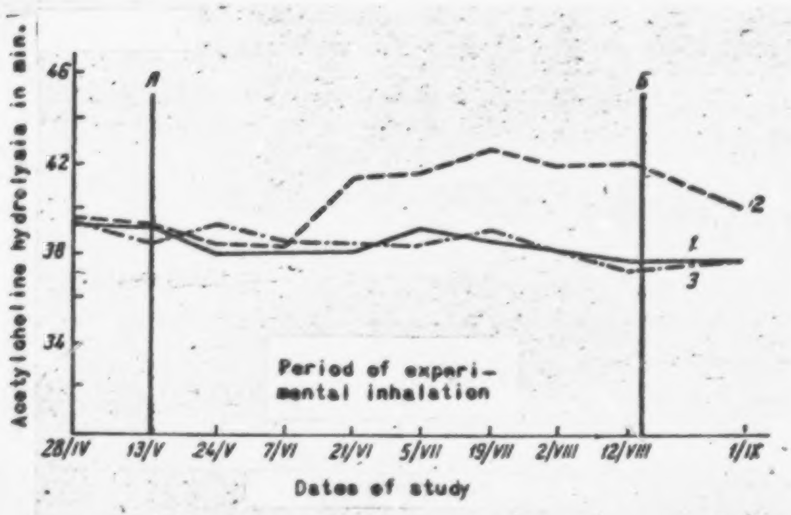

Fig. 4. Changes in whole blood cholinesterase activity in rats following methanol vapor inhalation. Notations same as in Fig. 2.

Changes appeared 7 weeks after the beginning of inhalation in rats of Group I. Results showed that albumin content dropped and the amount of beta- and gamma-globulins increased. Alpha-globulin content did not change. The fractional protein picture in rats of Group II did not change (Table 4).

Table 4

TOTAL PROTEIN AND PROTEIN FRACTIONS IN BLOOD SERUM IN RATS CHRONICALLY EXPOSED TO INHALATION OF METHANOL VAPOR

Inhalation period	Group	Total protein	Albumin	Globulins			Albumin/Globulin ratio
				Alpha	Beta	Gamma	
Before exposure	I	9,02	41,8	24,6	20,7	12,9	0,72
	II	9,31	44,8	23,2	19,4	12,6	0,81
	III	8,91	45,1	23,3	19,2	12,4	0,83
	Control						
On 45th inhalation day	I	8,79	34,7	24,0	24,0	17,3	0,53
	II	9,01	44,1	21,5	20,2	14,2	0,79
	III	9,02	43,6	20,9	20,9	14,6	0,87
	Control						
On 90th day	I	9,17	32,8	23,3	25,8	18,1	0,49
	II	9,16	42,0	21,8	21,9	14,3	0,72
	III	8,89	42,9	21,8	21,2	14,1	0,76
	Control						
On 20th recovery day	I	9,27	39,4	25,3	21,2	14,1	0,65
	II	9,05	42,2	20,2	22,1	15,5	0,73
	III	8,79	44,2	20,2	22,9	12,7	0,80
	Control						

Table 5

METHANOL VAPOR POLLUTED ATMOSPHERIC AIR IN THE ENVIRONS OF UZBEKISTAN HYDROLYTIC PLANTS

Meters from source of discharge	No. of samples	Single concentration in mg/m³		No. of air samples of concentrations	
		Maximal	Minimal	1 and higher	1 and below
THE ANDIZHANSK PLANT					
100	16	2,26	0,46	10	6
200	18	1,67	0,11	5	13
300	27	0,33	0,05	—	27
500	18	0,14	—	—	18
THE FERGANSK PLANT					
100	17	4,49	0,89	16	1
200	15	2,2	0,1	9	6
300	25	0,83	0,11	—	25
500	19	0,3	—	—	19
THE YANGIYULLSK PLANT					
100	14	1,15	0,1	3	11
200	24	0,55	1	—	24
300	14	0,05	—	—	14

No changes were observed in the total proteins. The results show that after 90 days only rats of Group I (5.31 mg/m^3)had been affected. A methanol-vapor concentration of 0.57 mg/m^3 was inactive. Accordingly 0.5 mg/m^3 is recommended as the average daily maximal permissible concentration.

An air pollution survey was conducted with reference to methanol in May and August 1962 in the proximity of the Andizhansk, Fergana, and Yangiyul' hydrolytic plants of Uzbekistan, leeward of the plants at distances of 100 to 500 from the discharge point (Table 5).

Data in the table show that at 100 and 299 m from the first two plants the methanol concentration in the air exceeded the allowable maximum (1 mg/m^3). At the third plant this excess was detected only at 100 m. At greater distances from the plants all concentrations were less than 1 mg/m^3.

CONCLUSIONS

1. Methanol air pollution in the vicinity of hydrolytic plants was detected up to 200 m from the plants.

2. The methanol odor perception threshold for highly sensitive individuals was determined at 4.5 mg/m^3, of eye sensitivity to light at 3.35 mg/m^3, and of effects on electrical brain activity at 1.17 mg/m^3.

3. It is, therefore, recommended that the maximum allowable methanol single exposure concentration in atmospheric air should not exceed 1 mg/m^3.

4. Chronic, 24 hr., 90-day inhalation of methanol in the air in a concentration of 5.3 mg/m^3 elicited in experimental rats changes in direct antagonist chronaxy ratio, in whole blood cholinesterase activity, in the elimination rate of coproporphyrin via the urine, and in the blood serum protein fractions. A concentration of 0.57 mg/m^3 proved inactive.

5. It is recommended that the average daily methanol concentration in the air be set at 0.5 mg/m^3.

ATMOSPHERIC AIR POLLUTION WITH VAPORS OF HYDROLYTIC ALCOHOL AND ITS EFFECT ON THE ORGANISM

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Hydrolytic alcohol is ethyl alcohol (ethanol) containing a variety of acid admixtures in 0.036-0.13 g/li concentration computed on the basis of acetic acid, unsaturated compounds in 0.07-0.89 g/li on the basis of allyl alcohol, 3-6.2 g/li of methanol, 1.7-21.6 g/li of carbonyl computed on the basis of acetaldehyde, 0.024 g/li of furfural and 2.3 g/li of higher spirits computed as isobutyl alcohol. Normal ethanol is a transparent, colorless, volatile fluid with a specific odor having a boiling point of 78.4° C. It mixes in all proportion with water, ether, and chloroform, and dissolves mineral salts, especially chlorides, nitrates, acetates, and some essential fatty oils. Ethanol is produced normally by fermentation from such starch-containing materials as potatoes, cereals, rice, or molasses. Synthetic ethanol is produced from ethylene and acetylene.

The most widespread of the ethanol products is hydrolytic alcohol, the manufacture of which is based on fermentation of hexose sugar obtained from hexose-containing plants. Ethanol is used as a solvent in the manufacture of varnishes and polishes, in synthesizing many organic compounds, as a raw material in the production of synthetic rubber by the Lebedev method, as a fuel for internal-combustion engines, and preparing food, medicines, and other items of the economy. Ethanol has narcotic properties and can be classed as a poison. Chronic use or exposure to ethanol has caused organic diseases of the nervous, digestive, and cardiovascular systems, liver, etc.

However, no information was found in the literature on the effects of low ethanol concentrations on man and animals as a result of vapor inhalation. Its maximum permissible concentration in atmospheric air has also not been established, and no data were found on air pollution around plants producing or using ethanol. These phases of air pollution were studied by the present author and discussed in this report.

Atmospheric air pollution with ethyl alcohol was determined by I. A. Pinigina's method (1961), which is based on the interaction of alcohol with a vanadium-hydroxyquinoline complex, which formed an orange dye. The sensitivity of the method is 0.002 mg in 2.5 ml. To obtain the required ethanol-air mixture the air was passed through an S-shaped device filled with hydrolytic ethyl alcohol. For several days before the experiment was initiated the ethanol concentration constancy in the cylinder was checked and found that its fluctuations were negligible. Threshold of odor perception was tested on 25 persons aged 18 to 40. Altogether 385 readings with 7 concentrations between 6.3-14.8 mg/m³ were made. Results are summarized in Table 1.

Table 1

THRESHOLD ODOR DETERMINATION OF HYDROLYTIC ETHYL ALCOHOL

Nos. of test persons	Concentrations in mg/m³	
	Minimal perceptible	Maximal non-perceptible
1	10,2	9,4
7	8,7	8,0
7	8,0	7,3
4	7,3	7,0
6	7,1	6,3

Methanol effect on eye sensitivity to light was checked next by the method of adaptation to darkness. Forty-two tests were made with persons aged 18-27 years and methanol concentrations of 6.12, 6.97, and 8.29 mg/m³. The lowest concentration was inactive in all four test persons; two responded to the middle concentration, and all reacted to the highest concentration (Table 2).

Table 2

THRESHOLD OF REFLEX CHANGES IN EYE SENSITIVITY TO LIGHT EFFECTED BY THE INHALATION OF HYDROLYTIC ETHYL ALCOHOL VAPOR, ON THE 20TH MINUTE EXPRESSED IN PERCENT OF THE 15TH MINUTE MAGNITUDES

Test persons' initials	Clean air	Concentration in mg/m³				
		8,29	6.97	6.12	Threshold	Subthreshold
I. L.	158,6 (o)	194,8 (b)	150,0 (o)	—	8,29	6,97
B. E.	156,0 (o)	226,7 (b)	155,6 (o)	—	8,29	6,97
Ch.A.	133,5 (o)	223,6 (c)	151,9 (b)	134,3 (o)	6,97	6,12
B.V.	148,0 (o)	214,4 (b)	130,7 (b)	147,8 (o)	6,97	6,12

Note: Reliability degree: a - 95%, b - 99%, c - 99.9%

The effect of low ethanol concentrations on the electrical activity of the cerebral cortex was studied by the A. D. Semenenko method (1963) of reflex-elicited alpha-rhythm rises in man, induced by simultaneous flashing of light stimulation at frequency approximating that of the rhythm and by a sound of variable intensity. On the background of such functional loading the natural alpha-rhythm became enhanced, and the nature of the curves recorded changed during inhalation of the gaseous mixture in active concentrations pointing to a change in the functional state of the nervous system and in the cerebral cortex activity. Curves were recorded on a 8-lead EEG manufactured by the Hungarian firm Orion Budapest. Test persons were five of the most sensitive to olfactory stimulation. Tests were conducted under identical conditions at the same time of the day. Four of five tests were made with each of the two test concentrations of 4.9 and 6.14 mg/m³. Statistical analysis of the results indicated that in all instances the lower ethanol concentration was inactive and the higher was active (Table 3).

Results are summarized in Table 4.

Table 3

EFFECT OF LOW HYDROLYTIC ETHYL ALCOHOL CONCENTRATIONS ON THE ELECTRICAL ACTIVITY OF THE BRAIN CORTEX

Test persons initials	6,1 mg/m^3		4.9 mg/m^3	
	Hemisphere		Hemisphere	
	Left	Right	Left	Right
I. S.	+	+	—	—
D. M.	+	+	—	—
R. K.	+	—	—	—
L. B.	+	+	—	—
M. K.	+	+	—	—

Note: + statistically significant; - statistically non-significant.

Table 4

EFFECT OF LOW HYDROLYTIC ETHYL ALCOHOL CONCENTRATIONS ON RESPIRATORY RECEPTIVE ORGANS

Functions	Concentration in mg/m^3	
	Threshold	Subthreshold
Odor perception	7,1	6,3
Eye sensitivity to light	6,9	6,1
Electrical activity of brain cortex	6,1	4,9

On the basis of results obtained with reflex reactions in man, it is proposed that 5 mg/m^3 of methanol in atmospheric air be adapted as the maximal allowable single hydrolytic ethyl alcohol concentration in atmospheric air.

In the next experimental series 45 white male rats weighing 100-120 g were divided into three groups and exposed to 24-hr., 90-day methanol air inhalation. Rats of Group II inhaled an ethanol-vapor concentration of 5 mg/m^3; rats of Group I-25 mg/m^3; and rats of Group III were the controls. Ethanol effects were evaluated on the basis of general behavior, gain or loss of body weight, motor chronaxy change, rate of coproporphrin elimination *via* the urine, whole blood cholinesterase activity, total amount of protein, and the protein fractions picture. Resulting numerical data were processed statistically.

The experimental ethanol-air mixture was fed into the chambers at a rate of (original sentence not complete), which V. A. Popov (1964) found to create the most favorable conditions for the animals. Actual ethanol concentrations were 29.25 ± 2.1 mg/m^3 for Group I and 5.59 ± 0.45 for Group II. During the inhalation period all animals were healthy, active, and gained body weight. However, rats of Group I manifested a negligible loss in body weight.

Muscle antagonist chronaxy was determined at the same hour of day and under identical conditions every 10 days in 5 rats of each group, employing an IES-01 pulse stimulator. Results are recorded in Table 5, Fig. 1. The data show that discoordination of the normal chronaxy ratio of flexor and extensor muscles in rats of Group I appeared during the 6th week of inhalation but the ratio returned to normal during the recovery period. Rats of Group II exhibited no statistically reliable changes as compared with the controls.

Table 5

MUSCLE ANTAGONISTS CHRONAXY RATIO IN RATS INHALING HYDROLYTIC ETHYL ALCOHOL VAPOR

Periods	Dates of study	Groups		
		I	II	Control Gp. III
		Extensor and flexor chronaxy ratios		
Before exposure	23/IV	1,62 (o)	1,53 (o)	1,65
	3/V	1,29 (o)	1,33 (o)	1,45
	13/V	1,30 (o)	1,43 (o)	1,58
During exposure	22/V	1,55 (o)	1,50 (o)	1,54
	31/V	1,26 (o)	1,58 (o)	1,48
	9/VI	1,37 (o)	1,53 (o)	1,53
	19/VI	0,82 (c)	1,42 (o)	1,41
	29/VI	0,93 (c)	1,49 (o)	1,54
	9/VII	0,91 (b)	1,35 (o)	1,57
	18/VII	1,1 (o)	1,42 (o)	1,44
	29/VII	0,91 (a)	1,26 (o)	1,44
	11/VIII	0,79 (o)	1,56 (o)	1,39
During recovery period	23/VIII	1,08 (o)	1,15 (o)	1,22
	1/IX	1,23 (o)	1,09 (o)	1,23

Note: Statistical reliability: a - 95%; b - 99.9%; c - not reliable

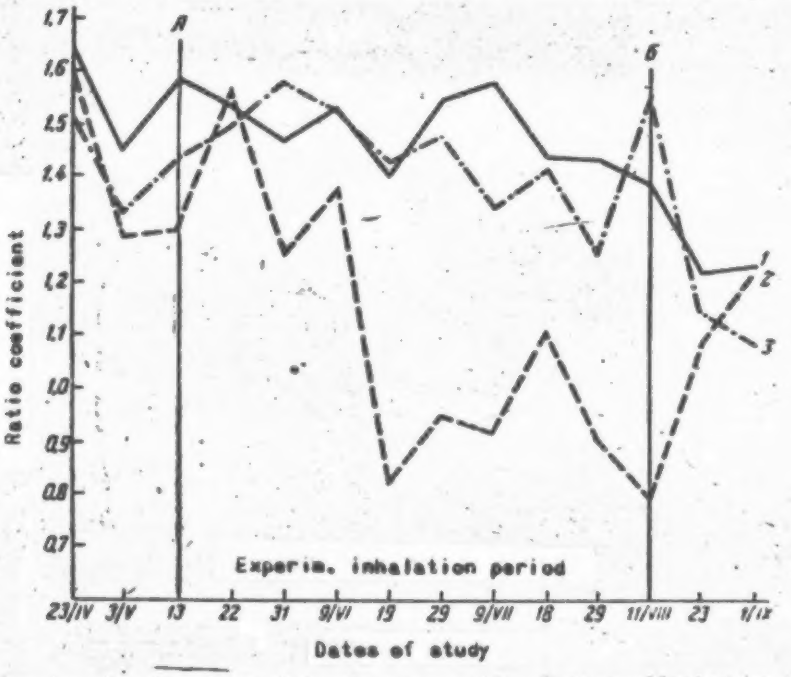

Fig. 1. Muscle antagonists chronaxy ratio changes effected by the inhalation of hydrolytic ethyl alcohol vapor.
A - B - Inhalation period; 1 - Clean air; 2 - 29.25 mg/m^3; 3 - 5.57/mg/m^3

Whole blood cholinesterase activity was tested in 5 rats of each group by the A. A. Pokrovskiy (1953) method and A. P. Martynova (1957) method. Blood was taken from the caudal vein once every 15 days. The average hydrolysis time was 37-39 min. (Table 6, Fig. 2). At the end of the 6th week rats of Group 1 exhibited a drop in whole blood cholinesterase activity, acetycholine time rose to 42 min. However, at the end of the 9th week of inhalation it normalized, only to rise again during the 10th and 11th weeks. This increase was evidently related to the protective or defense function of the animals' organism. After 20 days of recovery hydrolysis rate returned to normal. The rats of Group II evidenced no changes as compared with the control group.

Table 6

CHANGES IN RATS' CHOLINESTERASE ACTIVITY DURING HYDROLYTIC ETHYL ALCOHOL VAPOR INHALATION IN MINUTES OF ACETYLCHOLIN HYDROLYSIS

Periods	Dates	Groups		
		I (29.2 мг/м³)	II (5.59 мг/м³)	Control III
Before exposure	28/IV	37,6 (o)	37,0 (o)	39,2
Inhalation	13/V	37,8 (o)	38,8 (o)	39,2
	24/V	39,8 (o)	34,4 (o)	38,0
	7/VI	39,0 (o)	39,2 (o)	38,0
	21/VI	42,0 (c)	38,6 (o)	38,2
	5/VII	42,2 (b)	38,8 (o)	38,8
	19/VII	39,8 (o)	37,4 (o)	38,8
	2/VIII	41,8 (в)	37,8 (o)	38,0
	12/VIII	42,2 (a)	37,8 (o)	37,6
Recovery	1/IX	38,7 (o)	38,0 (o)	37,4

Note: Reliability degree: a - 95%; b - 99%; c - 99.9%, o - nonreliable

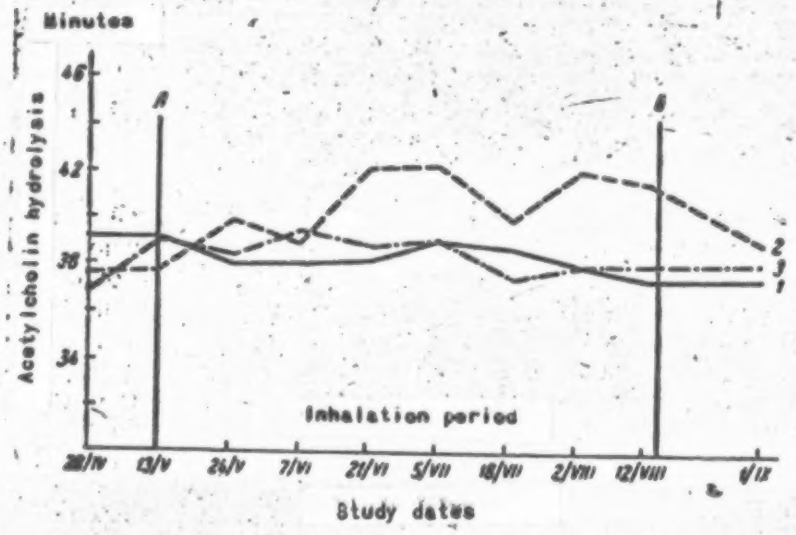

Fig. 2. Changes in rats' cholinesterase activity during inhalation of hydrolytic ethanol vapor. Notations same as in Fig. 1.

Effect of low ethanol vapor concentrations on the organism's metabolic processes was evaluated on the basis of porphyrin metabolism as recommended by Fisher. Porphyrin was extracted from the urine with ether and determined photocolorimetrically. Twenty-four hour urine specimens of 5 rats were used in the combined determinations.

Rate of coproporphrin elimination was checked fortnightly and recorded on the basis of 100 g of body weight. In rats of Group I the excretion rate dropped sharply during the 6th week and remained at this level through experimental inhalation period (Fig. 3). At the end of the recovery period the level returned to normal. Animals of Group II manifested no statistically reliable changes.

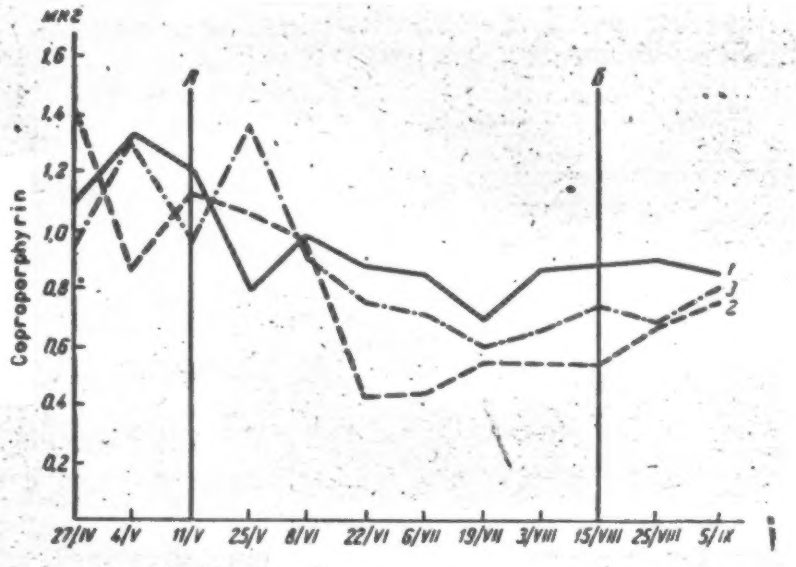

Fig. 3. Changes in rate of coproporphyrin elimination via the urine of rats inhaling hydrolytic ethanol vapor. Notations same as in Fig. 1.

Paper electrophoresis was employed in studying the effects of low ethanol vapor concentrations on total protein and its fractional composition. The blood was taken from the caudal vein on an empty stomach every 15 days. At the end of the second month of inhalation the albumins in rats of Group I fell and the gamma-globulin fraction increased. This increase was statistically reliable and persisted to the end of the experiment. The albumin/globulin coefficient did not return to normal during the recovery period (Table 7, Fig. 4). No changes were observed in rats of Group II. None of the experimental animals showed any change in the total protein.

In summary it can be stated that continuous 90-day inhalation had a marked effect on animals of Group I (29.1±2.1) and none on those of Group II (5.59±0.045 mg/m^3). On this basis it is recommended that the allowable maximum single-exposure concentration of methanol in atmospheric air be set at 5 mg/m^3; the same limit should be adapted for the average daily concentration of hydrolytic ethyl alcohol.

A study was made of the distribution of methanol (hydrolytic ethyl alcohol) air pollution in the vicinity; 186 samples of air at 100, 200, and 300 m from three hydrolysis plants in Uzbekistan, always on the leeward side of the emission source, were collected. The samples were aspirated through two activated-coal absorbers. Air samples varied from 10-40 liters, aspirating the air at the rate of 1 li/min., at air temperature of 20 to 35° C, relative humidity from 25 to 70 percent, barometric pressure from 730 to 750 mm Hg, wind velocity from 1 to 5 m/sec. The weather was arid and clear (Table 8).

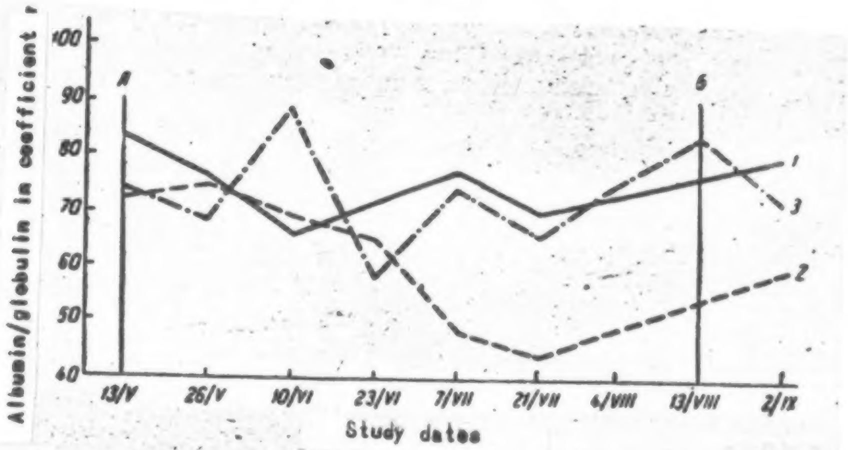

Fig. 4. Changes in blood serum protein fractions of rats inhaling hydrolytic ethanol vapor
Notations same as in Fig. 1.

Table 7

CHANGES IN BLOOD SERUM TOTAL PROTEIN AND PROTEIN FRACTIONS OF RATS DURING INHALING HYDROLYTIC ETHANOL VAPOR

Period	Groups	Total protein	Albumins	Globulins			Albumin/globulin coefficient
				Alpha	Beta	Gamma	
Prior to ethanol inhalation	I	9,09	42,3	23,8	19,5	14,4	0,73
	II	9,05	42,6	22,9	20,4	14,1	0,74
	III	8,91	45,1	22,9	19,4	12,6	0,83
	Control						
On 50th inhalation day	I	8,89	32,3	24,6	26,0	17,1	0,48
	II	9,04	42,6	21,6	21,4	14,4	0,74
	III	9,88	43,7	20,8	20,9	14,6	0,77
	Control						
On 90th inhalation day	I	9,40	36,3	23,4	23,5	16,8	0,54
	II	8,63	45,4	21,1	21,1	12,4	0,84
	III	8,73	42,9	21,8	21,1	14,2	0,76
	Control						
During recovery period	I	9,38	37,9	24,0	22,6	15,5	0,60
	II	8,67	44,8	21,1	21,8	12,3	0,83
	III	8,79	44,2	19,8	23,4	12,6	0,80
	Control						

Table 8

ATMOSPHERIC AIR POLLUTION WITH HYDROLYTIC ETHANOL VAPOR IN THE ENVIRONS OF THE UZBEKISTAN PLANTS

Meters from discharge point	No. of air samples	Single concns. in mg/m^3		Number of samples with indicated concns.		
		Maximal	Minimal	2 and higher	1 to 2	Below 1
			Fergansk plant			
100	26	2,79	0,55	6	9	11
200	17	0,16	—	—	—	17
300	27	0,018	—	—	—	27
			Andizhansk plant			
100	25	4,52	0,45	10	10	5
200	16	1,68	—	—	2	14
300	27	0,37	—	—	—	26
			Yanguiyulsk plant			
100	44	2,2	—	4	10	10
200	25	0,5	—	—	—	25

Data in Table 8 show that emissions at 100 m or more from the three plants under survey contained ethanol concentrations below the recommended limit for single-exposure (5 mg/m^3).

CONCLUSIONS

1. Air around the investigated Uzbek hydrolytic plants contained negligible concentrations of hydrolytic ethyl alcohol.

2. The present study showed that the thresholds of ethanol odor perception in most sensitive persons was 7.1 mg/m^3; eye sensitivity to light was 6.97 mg/m^3; and of reflex effect on cerebral cortex activity 6.1 mg/m^3. The maximum inactive or subthreshold concentration in most sensitive persons was 4.9 mg/m^3.

3. It is recommended that the maximum allowable single concentration of hydrolytic ethyl alcohol in atmospheric air be set at the 5 mg/m^3 level.

4. Chronic, 24-hr, 90-day inhalation by experimental rats of air containing ethanol vapor in 29.25 mg/m^3 concentration affected the flexor extensor muscle chronaxy ratio, the cholinesterase activity, the rate of coproporphyrin elimination via the urine, and the ratios of blood-serum protein fractions. A 5.59 mg/m^3 ethanol concentration had no effect on the organism.

5. Results of chronic experiments with rats indicated that the maximal allowable average 24-hr. ethanol vapor concentration in atmospheric air can be set at 5 mg/m^3.

BIOLOGICAL EFFECTS AND HYGIENIC EVALUATION OF AIR BY PHTHALIC ANHYDRIDE

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From the Ukrainian Scientific-Research
Institute of Communal Hygiene

Phthalic anhydride (PhA) is a benzene ortho-dicarboxylic acid anhydride. Its boiling and melting points are 284.5 and 128-131° C respectively; its odor resembles that of bitter almonds. It sublimates easily and dissolves in water (0.6 g in 100 ml at 25° C). When dissolved in hot water it converts to phthalic acid. It is also soluble in sulfuric acid, alcohol, ether, and benzene. PhA sublimates in the form of flat, white often rhombic crystals. PhA is used in many branches of industry. It is widely used in dye production: phthaleins, rhodamines, indigo, anthranilic acid, derivatives of anthraquinone, and phthalocyanines. Orthophothalic acid esters are used as plasticizers in producing glyptal, polyester, and epoxy resins. It is also used in the pharmaceutical and tanning industries, and because of its wide reactive capacity it is used in chemical laboratories.

PhA can be manufactured in several ways, the most common industrial of which is based on oxidizing naphthaline in the presence of a catalyzer at 350-500°C. The manufacture and use of PhA are factors associated with air pollution. After cleaning in scrubbers, waste gases from PhA production are discharged into the air, part of the PhA being discharged by the plants' ventilators. No references were found in the literature dealing with the existence of PhA in the air around industrial enterprises. Effects of PhA on the human and animal organism have been studied previously; and some authors (Friebel et al., 1956; L. A. Titunov, A. A. Denisenko, 1957; Yu. K. Korotkova, 1957, 1960; S. N. Kremneva, M. S. Tolgskaya, 1961) investigated the toxicity of PhA when introduced into the gastroenteritic tract. S. N. Kremneva and M. S. Tolgskaya (1961) administered a 2 percent emulsion of PhA to narcotized white rats intratracheally. Doses in excess of 30 mg/kg were lethal, death ensuing in a few hours or in the course of days. Friebel, et al. (1956) investigated inhalation of PhA dust and vapor by guinea pigs in concentrations of 644.5 mg/m^3, respectively. These concentrations irritated the conjunctiva and respiratory passages. An admixture of naphthoquinone and maleic acid enhanced the irritating effect.

In 1959 Yu. K. Korotkova subjected 10 guinea pigs to the inhalation of PhA dust (600 mg/m^3) every day for 15 min. for 30 days. The concentration was determined gravimetrically. The animals coughed and sneezed during the inhalation tests; and after the first inhalation three of the animals exhibited palpebral edema, conjunctival hyperemia, and nasal discharges. Body weight loss was observed in a few cases. Dissection disclosed hyperemia of the mucous membrane of the trachea and of the bronchi accompanied by fusing of the pleural surfaces and moderate plethora of the internal organs. S. N. Kremneva and M. S. Tolgskaya (1961) conducted static injection experiments for 6 months, 6 days a week, 3 hrs. a day, with PhA of 5-12 mg/m^3. At the end of the 6th month arterial pressure had dropped to 70 percent of its normal level. Eosinophilia developed in the majority of cases in the course of injections. Results showed that a 1-2 mg/m^3 concentration was ineffective. The review shows that only high PhA concentrations were studied for brief periods. The effect of low concentrations for 24 hr. periods has not been investigated. Furthermore, a maximal allowable limit of 1 mg/m^3 of PhA has been set for industrial premises, none has been established for atmospheric air.

The wide use of PhA by the industry, the insufficient study devoted to its toxic properties, and the prevailing local air pollution sources prompted the present author to study the problem of atmospheric air pollution with PhA. The purpose of the present paper is to find an experimental basis for a maximal allowable PhA concentration in atmospheric air. Here as in other air pollution studies, the thresholds of odor perceptions and of effects on eye sensitivity to light were investigated first. The M. D. Manita spectrophotometer was used in the study.

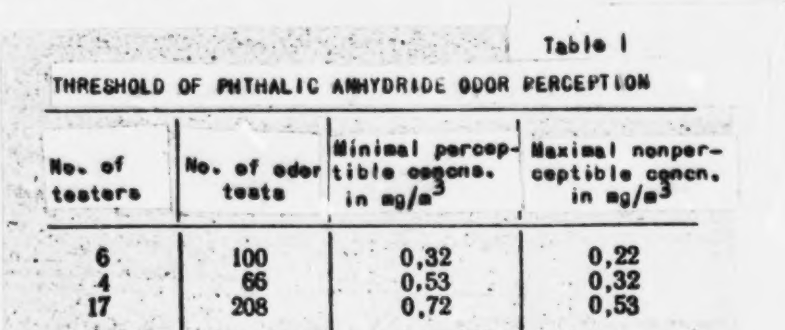

Table I

THRESHOLD OF PHTHALIC ANHYDRIDE ODOR PERCEPTION

No. of testers	No. of odor tests	Minimal perceptible concns. in mg/m^3	Maximal nonperceptible concn. in mg/m^3
6	100	0,32	0,22
4	66	0,53	0,32
17	208	0,72	0,53

The odor perception threshold was determined on the basis of 374 observations and 27 healthy test volunteers. Results are listed in Table 1, and show that the highest nonactive PhA concentration range was 0.32-0.72 mg/m^3.

Recent studies aimed at determining maximal single exposure concentration of PhA, established, by tests of eye sensitivity to light, 0.22 mg/m^3 as the maximal allowable concentration. Effect on PhA on eye sensitivity to light was investigated in cooperation with three test persons using adaptometer ADM making determinations at 5 min. intervals. Results are listed in Table 2. The 0.96 mg/m^3 concentration elicited statistically reliable changes on the 20th minute of dark adaptation of all test persons.

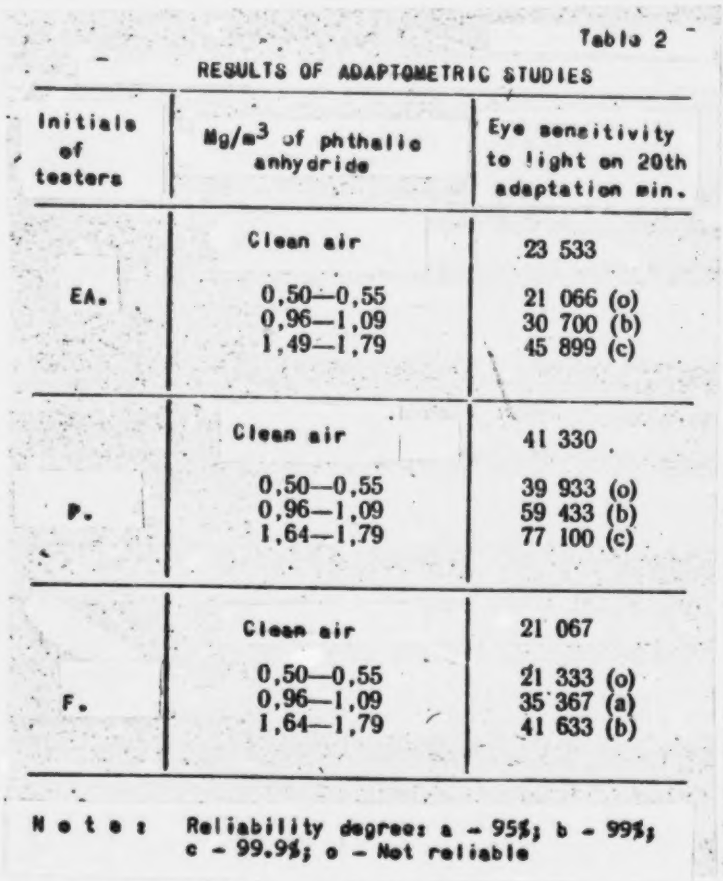

Table 2

RESULTS OF ADAPTOMETRIC STUDIES

Initials of testers	Mg/m^3 of phthalic anhydride	Eye sensitivity to light on 20th adaptation min.
EA.	Clean air	23 533
	0,50—0,55	21 066 (o)
	0,96—1,09	30 700 (b)
	1,49—1,79	45 899 (c)
P.	Clean air	41 330
	0,50—0,55	39 933 (o)
	0,96—1,09	59 433 (b)
	1,64—1,79	77 100 (c)
F.	Clean air	21 067
	0,50—0,55	21 333 (o)
	0,96—1,09	35 367 (a)
	1,64—1,79	41 633 (b)

Note: Reliability degree: a - 95%; b - 99%; c - 99.9%; o - Not reliable

The 0.55 mg/m^3 concentrations caused statistically reliable changes on the 20th minute of adaptation to the dark. The 0.55 mg/m^3 concentration was inactive. In two of the three test persons 0.72 mg/m^3 of PhA was the threshold of odor perception. No such test was made with the third person.

On the basis of effects which PhA yielded in the trigeminal tests, it is recommended that 0.2 mg/m^3 PhA concentration in atmospheric air be set as its allowable maximal single concentration.

The allowable maximal average daily concentration was determined by using 60 white male rats enclosed in 100-liter chambers into which prefiltered air was run in at the rate of 20-23 li/min. Rats were exposed to 24 hour daily inhalations for 70 days. Rats were divided into four groups which inhaled air containing the following PhA concentrations in mg/m^3: rats of Group I--1.52; (misprint for 1.32--B.S.L.); rats of Group II--0.54; of Group III--0.18; rats of Group IV served as controls. It should be noted that the Group I concentration is close to the allowable level in industrial premises; 0.18 is below the odor perception threshold and close to the recommended single-exposure concentration; 0.54 is a concentration often encountered in the air at 500 m from PhA plants located in the proximity of inhabited areas.

During the chronic experiment records were kept of the rats' condition, their body weight, muscle antagonists, motor chronaxy, cholinesterase activity, and the morphological composition of the blood. The behavior and activity of the rats in the first three groups did not differ from that of the controls. The rats in all groups gained weight.

It has been shown by many authors that changes in motor chronaxy ratios reflected changes in the functional state of the central nervous system (Yu. N. Uflyand, 1941; A. N. Magnitskiy, 1948; A. F. Makarchenko, 1956). This index has become first in the order of studies conducted in determining limits of allowable maximal air pollutant concentrations in atmospheric air. It was used for the purpose in the present studies. This index was also used in experiments with animals, in 5 rats of each group by a technique and procedure described in some of the preceding reports. Deviations from the normal (control) muscle antagonist chronaxy ratios in rats of Group I were noted on the 31st day of the experiment; two weeks later chronaxy ratios returned to normal. Rats of Group I manifested less pronounced changes, and those of Group III showed no chronaxy ratio changes (Fig. 1).

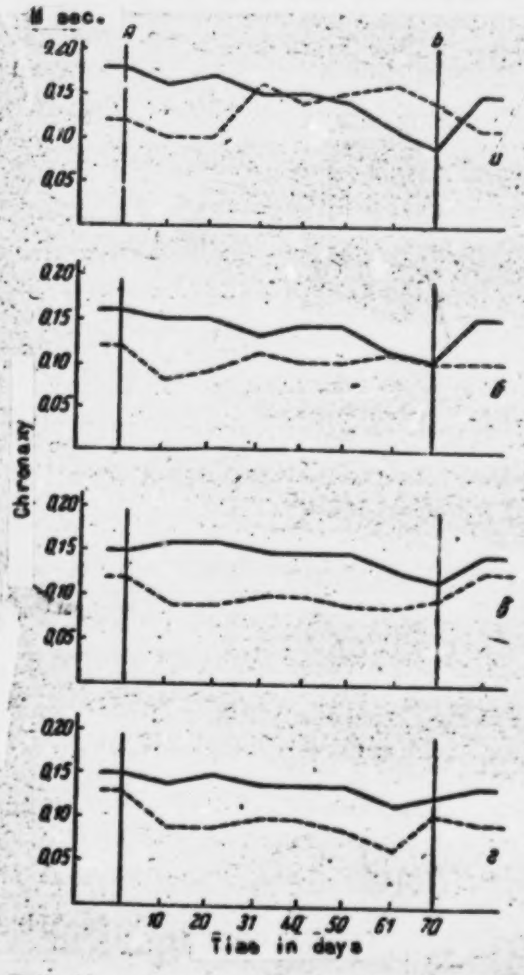

Fig. 1. Phthalic anhydride effect on muscle antagonists motor chronaxy of Group I rats (a), Group II (b), Group III (c), and Group IV rats (d). Solid line curves - extensors, broken line curves - flexors
AB - Inhalation period

Previous studies have indicated that cholinesterase activity changed in some pathological conditions of the organism, giving rise to an index utilized in arriving at hygienic norms. G. I. Solomin (1961), V. A. Chizhikov (1963), F. I. Filatova (1962), and D. G. Odoshashvili (1962) demonstrated that blood cholinesterase activity was affected by chemical substances.

The present author studied cholinesterase activity of whole blood by the chemical method of A. A. Pokrovskiy (1953) as modified by A. P. Marynova (1956). Cholinesterase activity was evaluated on the basis of change in acetylcholine hydrolysis time. Rats of each group were examined once every 2 weeks. On the 42nd day of administration, rats of Group I experienced inhibition of cholinesterase activity, acetylcholine hydrolysis time rising to 49 min. (from 41). Statistically reliable changes were noted in rats of Group II, but they were less pronounced. Hydrolysis time in rats of Group III did not vary from that of the controls (Fig. 2).

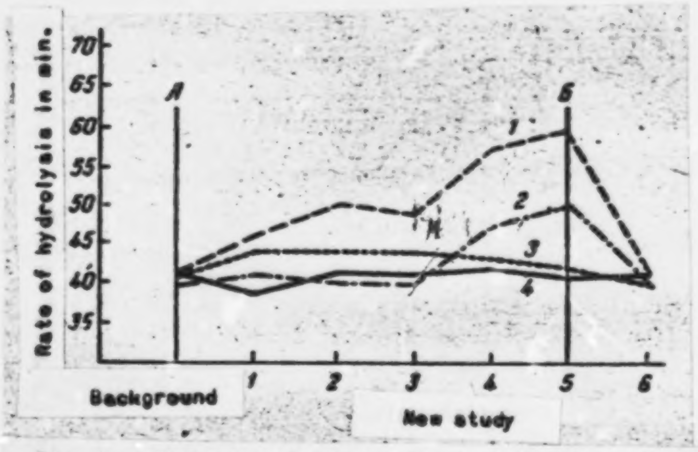

Fig. 2. Phthalic anhydride effect on blood cholinesterase activity

1 - Group I; 2 - Group II; 3 - Group III; 4 - Group IV (Control). AB - Inhalation period

Evidence presented in the literature that PhA affected the hematopoetic system (K. Ye. Bakaleynik, 1960; S. N. Kremneva and M. S. Tolgskaya, 1961; V. S. Anatovskaya, 1961). This fact served as the basis for analyzing the morphological composition of the blood. Determinations were made of the amount of hemoglobin, erythrocytes, thrombocytes, and leucocytes. Changes in the thrombocyte count in rats of Groups I and II appeared on the 70th day. No substantial changes were found in leucocyte count. Hemoglobin and erythrocytes fluctuated within normal limits. Thus, the results indicated that the chronic experiments brought into evidence of changes in the motor-chronaxy ratios of the muscle antagonists, cholinesterase activity, and thrombocyte counts in rats of Group I (1.32 mg/m^3). Less pronounced changes appeared later in the study in rats of Group II. During the 2-week recovery period these functions returned to their normal levels. No deviations were noted in rats of Group III. Data of the chronic experiments were processed statistically and confirmed the conclusions. On the basis of results obtained in this study it is recommended that 0.2 mg/m^3 should be adapted as the maximal allowable PhA in atmospheric air. A survey was made of air pollution with PhA plants producing it using the I. B. Kogan (1961) polarographic method and aspirations containing 96° ethylol.

The possibility of PhA vapors and aerosol escaping into the plant surrounding atmosphere was checked. The plant produced 10,000 tons of PhA per annum. Table 3 presents data on the level of pollution by PhA vapors and aerosols. Results showed that only at 1000 m from the plant did the sample concentrations fall below the 0.2 mg/m^3 allowable concentration.

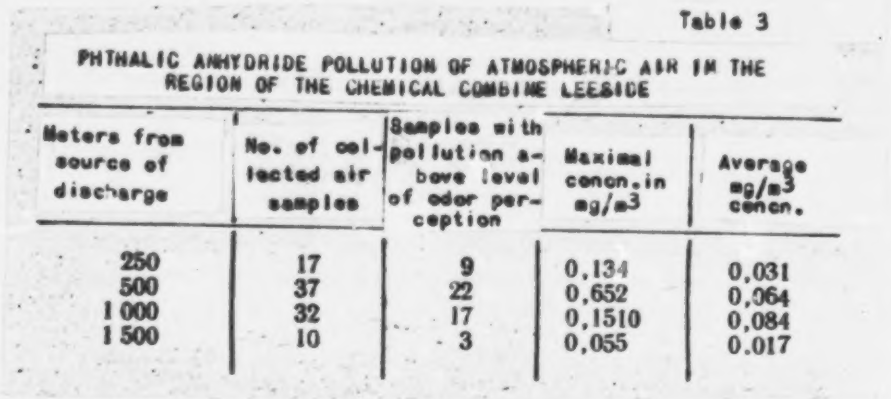

Table 3

PHTHALIC ANHYDRIDE POLLUTION OF ATMOSPHERIC AIR IN THE REGION OF THE CHEMICAL COMBINE LEESIDE

Meters from source of discharge	No. of collected air samples	Samples with pollution above level of odor perception	Maximal concn. in mg/m^3	Average mg/m^3 concn.
250	17	9	0,134	0,031
500	37	22	0,652	0,064
1 000	32	17	0,1510	0,084
1 500	10	3	0,055	0.017

For PhA producing plants the sanitary break zone should be not less than 1000 m. The zone proposed by construction planners (500 m) is of insufficient width for plants with a production capacity of over 10,000 T/year.

CONCLUSIONS

1. The threshold of phthalic anhydride odor perception is 0.32 mg/m^3 for most sensitive persons; 0.22 mg/m^3 is the subthreshold concentration.

2. Analysis of the dark-adaptation curve for short-time inhalation of PhA indicated that its activity threshold is 0.92 mg/m^3, while 0.55 is a subthreshold concentration.

3. 0.2 mg/m^3 is recommended as the maximum single-exposure concentration of PhA.

4. Chronic, 24 hr., 70-day exposure to inhalation of vapors and aerosols of a PhA in concentrations of 1.32 and 0.54 mg/m^3 elicited statistically reliable changes in the flexor and extensor chronaxy ratio in whole blood cholinesterase activity and in the thrombocyte count. A 0.2 mg/m^3 PhA concentration proved inactive and is recommended as the average daily maximal concentration.

5. For reasons discussed in a preceding paragraph of this paper, provision should be made for 1000 m sanitary break zones around plants built for the production of 10,000T of PhA per annum.

COMPARATIVE TOXICITY STUDIES OF BENZENE, TOLUOL, AND XYLOL BY THE REFLEX ACTIVITY METHOD

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Aromatic hydrocarbons benzene, toluol, and xylol have been studied by toxicologists and hygienists for many years past. Despite the facts that these substances have been and still are extensively used industrially and that they all possessed broad toxic spectra, the information found in the literature regarding their comparative toxicity, no allowable limits have been established for their concentration in the living environment.

Pure benzene, toluol, and xylol are colorless liquids having specific aromatic odors, and all are poorly soluble in water. The boiling point of benzene is 80.1°, of toluol--110.8°, and of xylol--140.5°. Benzene is three times, toluol 4.2 times, and xylol 1.35 as volatile as ether. All are good solvents of fats, resins, raw rubber, essential oils, and dyes and other organic compounds. These compounds are widely used as solvents in different industries. Benzene is also widely used in organic synthesis of phenol, nitrobenzene, chlorobenzene, maleic anhydride, and other compounds. Toluol is a raw material used by the explosives industry. Xylol is used in the production of xylidine and of phthalic acid.

Benzene, toluol, and xylol have a narcotic effect primarily on the nervous system. Thus, G. E. Rozentsvit (1954) showed that functional disruptions of the CNS led to morphological changes in the blood. Some investigators believe that changes in other organs were the causes of nervous and endocrine systems poisoning. Changes in reflex activity in animals at various concentrations of benzene have been reported by R. I. Yaroslavskaya and I. M. Rozovskiy (1952), Yu. V. Novikov (1957), A. I. Korbakova, S. I. Kremneva, N. K. Kulagina and I. P. Ulanova (1960). A. A. Golubev (1959) injected mice with xylol in 550 mg/m^3 concentration and observed delays in conditioned reflex reactions and delayed differentiation response. Ye. N. Lyublina (1950) exposed rabbits for 40 min. to benzene, toluol, and xylol to test their effect on the CNS. The concentrations were for benzene and toluol 300, 400, 1000 mg/m^3, and for xylol 100, 200, 400 mg/m^3. Concentrations which broke down unconditioned reflexes in rabbits were: benzene 1500, toluol 1000, and xylol 750 mg/m^3. (A. S. Faustov, 1961). The author showed that xylol, unlike benzene and toluol, acted as a CNS depressant.

Comparative evaluation of the toxicity of benzene, toluol, and xylol have been conducted by different authors based on their effects on blood and the hematopoeitic organs. From this point of view (M. G. Mgebrov, N. D. Rozenbaum, Lind and others) benzene was the more toxic. Others believed that chronic toluol and benzene poisoning depressed the CNS activity equally. R. G. Leytes, B. I. Martsinkovskiy and L. K. Khotsyanov are of the opinion that toluol and xylol acted analogously to benzene, but produced significantly fewer changes in the blood (O. N. Olimpiyeva, V. M. Retnev, A. P. Rusinova, 1958). Studies and comparative evaluation of effects of these compounds in minimal effective concentrations are of basic interest toxicologically and from the standpoint of sanitary production of atmospheric air.

Scanty information was found in the literature on air pollution by benzene, toluol, and xylol. Major production and processing industries are not the only air pollution sources. Discharges from small industries also played an important contributing part. Having specific odors, the compounds under study affected nasal and upper respiratory receptors and elicited series of reflex reactions in which higher nervous centers and the cerebral cortex played a part. Odors and reflexes elicited by them are not without sequential effects. As cortical reflexes they have the capacity to form temporary interconnections with a variety of processes (S. V. Anichkov, 1952).

Recent studies have shown that threshold concentrations of chemicals could induce reflex reactions such as change in vascular tonus, rhythms and depth of pulmonary response, optimal chronaxy, skin-galvanic response and eye sensitivity to light (K. A. Bushtuyeva, 1960; M. K. Borisova, 1960; M. T. Takhirov, 1960, and others), with or without accompanying recordable cortical responses. Because of this it is possible to study the threshold of odor perception of these compounds, which have not been studied before. Such studies were made by different methods. Thus, N. V. Lazarev (1963) determined odor perceptions thresholds for benzene at 5 mg/m^3, for toluol at 2 mg/m^3, and for xylol at 0.8 mg/m^3. According to Yu. V. Novikov (1956) the odor perception threshold for benzene is 3 mg/m^3. Chen' Yun'-tay set the odor perception threshold of orthoxylene at 0.73 mg/m^3 (1963).

The present author determined odor perception thresholds by the method proposed by the Committee on Sanitary Protection of Atmospheric Air (V. A. Ryazanov, K. A. Bushtuyeva, Yu. V. Novikov, 1957). Benzene, toluol, and xylol concentrations were determined by the method of M. V. Alekseyeva (1964). The method is based on the nitration of the compounds followed by the extraction of the nitrogen compounds with butanol. Upon the addition of alkali to the extract a color appeared (for benzene--violet; toluol--orange; metaxylol-green-blue). The colored solutions were read colorimetrically. Determinations were made with a FEKN-57 photoelectric colorimeter, and results compared with calibrated standards. The sensitivity of the method is 0.5 micrograms for benzene, 1 microgram for toluol, and 2 micrograms for xylol with 2 ml samples.

Table 1

DETERMINATIONS OF BENZINE THRESHOLD ODOR PERCEPTION

Number of observations	Mg/m^3 of benzene	
	Minimal perceptible	Maximal non-perceptible
8	>4,0	—
3	3,4	3,2
2	3,2	3,0
5	2,8	2,5

The odor perception threshold was determined with the cooperation of 18 test persons. In all 7 concentrations and 518 observations were studied. (Results are listed in Table 1).

The first benzene concentration studied was 4 mg/m^3; 8 of the 18 test persons found this concentration inactive; the minimal perceptible concentration for 5 test persons was 2.8 mg/m^3, and the maximal nonperceptible concentration was 2.5 mg/m^3. Thus, results of the experiments confirmed those obtained by Yu. V. Novikov in 1956 (3 mg/m^3).

Studies of odor perception thresholds for toluol were made on 30 test persons, making a total of 744 tests and using 7 concentrations. Results are shown in Table 2.

Table 2

DETERMINATIONS OF TOLUOL VAPOR THRESHHOLD ODOR PERCEPTION

No. of determinations	Mg/m^3 of toluol	
	Minimal perceptible	Maximal non-perceptible
5	>3,2	—
6	3,2	2,8
4	2,8	2,3
3	2,3	2,0
3	2,0	1,8
3	1,8	1,5
6	1,5	1,27

Five of the 30 test persons failed to detect the odor of toluol in concentration of 3.2 mg/m^3; for 6 persons 1.5 mg/m^3 was the minimum perceptible concentration for most sensitive persons. The maximal nonperceptible concentration for these persons was 1.27 mg/m^3. The odor perception threshold of 1.5 mg/m^3 for toluol agreed with the data of N. V. Lazarev (2 mg/m^3).

Tests for determination of metaxylol odor perception threshold were made on 18 test persons using 6 concentrations with 431 observations in all. Results are shown in Table 3. Data in Table 3 show that the minimal perceptible concentration for the four most sensitive persons was 0.6 mg/m^3, and the maximal subthreshold concentration was 0.41 mg/m^3. Accordingly the odor perception threshold of metaxylol (0.6 mg/m^3) is almost identical with the concentration recorded by Chen' Yun'-tay (0.73 mg/m^3). Accordingly, the drop in the odor perception threshold concentration from benzene to xylol was already demonstrated inactive, that an increase in the number of methyl groups enhanced the odor intensity in this chemical series.

Table 3

DETERMINATION OF XYLOL VAPOR THRESHOLD ODOR PERCEPTION IN MG/M^3

No. of tests	Mg/m^3 of xylol	
	Minimal perceptible	Maximal non-perceptible
2	1,9	1,4
3	1,4	1,0
5	1,0	0,85
4	0,85	0,6
4	0,6	0,41

The next study aimed at determining the effects of their minimal concentrations affecting electrical activity of the cerebral cortex. Corticopotential rhythms reflected active processes in different sections of the cerebral hemispheres and other active phenomena in the CNS.

K. A. Bushtuyeva, Ye. F. Polezhayev, and A. D. Semenko (1960) first used the functional electroencephalographic method in their study of responses to reflexes evoked by minimal concentrations of chemicals. Their results showed that subthreshold odor perception concentrations alone elicited no visible effects, but altered electrical activity of the cerebral cortex. Recently, modifications of this method have been used in the study of effects of minimal concentrations of chemicals on the functional activity of the CNS. The present author determined the effect of different odor perception concentrations of benzene, toluol, and xylol by the method of quantitative analysis of alpha-rhythm reflex responses (A. D. Semenko, 1963) which it was thought best reflected the course of the cerebral cortex processes. The method is based on the entrancement of biopotentials induced by disturbances in the cortical electrical rhythms (trigger stimulation); it was proposed by Walter and Shipton (1949). N. P. Bekhtereva and V. V. Usov (1960) showed that trigger stimulation strengthened alpha-rhythms and was highly effective at high functional loads. In the present studies stimulation was induced by light pulsations of regulated intensity applied at five-second intervals. The effect of low concentrations of the substances on the elctrical brain activity had been recorded after the pattern of alpha-rhythms in the test persons was established. Experiments were conducted in a special dimly lit chamber. The test person was seated in a semirecumbent position in a comfortable chair. In front of him was a cylinder filled with fresh air which was released at the rate of 30 li/min.; at the proper moment the tested gas was mixed with the fresh air at desired concentrations; appropriate regulating and controling instrumentation was used in such experiments.

Ten observation tests were made with each tested pollutant concentration intermittently, five with the gas vapor and air and 5 with clean air. Each test consisted of 18 one-minute cycles consisting of 10 sec. of accoustical stimulations, 7 seconds free from stimulation, 18 sec. of stimulation with light and 25 sec. of active warm-up prior to recording simple rhythmic sterotypes and maintenance of general tonicity. Eighteen minutes of the experiment consisted of 3 minutes of training, 3 minutes to set up the experiment, and the remaining time for the experiment proper. Gaseous mixture was fed for 6 minutes, 6 minutes were given to recovery. The vapor-air mixture was released before initiating sound stimulation and ended with stimulation with light.

Analysis of the biocurrent records was carried out by integrating the energy of amplified rhythms. Results were processed statistically to determine reliability index changes. Five observations were made to determine concentration of subthreshold odor perception of benzene and its effect on the elctrical cerebro-cortical activity in most sensitive persons. Bipolar biocurrents were recorded on a 16-lead ECG manufactured by the Galileo company, from the temporal, parietal, and frontal regions individually and in combinations. Benzene concentration of 2 mg/m^3 produced enhanced electrical potentials from the left occipital-temporal part of the cerebral cortex. Dynamics of the indicated changes are shown in Table 4.

Table 4

RELIABILITY OF ELECTROPOTENTIAL BRAIN CHANGES EFFECTED BY THE INHALATION OF AIR CONTAINING 2 mg/m^3 OF BENZENE VAPOR

Initials of Testers	Air-gas mixture			Recovery period		
	Minutes			Minutes		
	1–2	3–4	5–6	1–2	3–4	5–6
A. S.	o	b	c	a	a	o
K. S.	o	a	c	o	o	o
M. Z.	o	a	b	o	c	o
N. V.	o	c	o	b	b	o
S. L.	o	b	c	c	c	o

Notes - Reliability: a - 95%; b - 99%; c - 99.9%; 0 - Not reliable

Data in Table 4 indicate that statistically reliable data were recorded during the 3rd-4th minute after inhalation of the gas mixture was initiated, increased by the 5-6th minute and disappeared by the 5-6th minute of the recovery period. Statistical treatment of data recorded from other parts of the brain showed no statistically reliable changes. Below the 1.5 mg/m^3 concentration, benzene produced no statistically reliable changes in electrical brain activity. Changes in bioelectrical potentials in test person N at different benzene concentrations are shown in Figure 1.

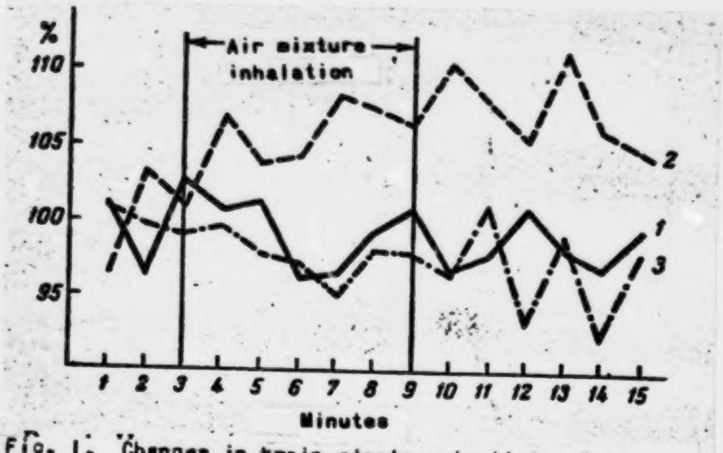

Fig. 1. Changes in brain electropotentials of test person effected by inhalation of different benzene concentrations
1 - Clean air; 2 - mg/m^3 of benzene; 3 - 1.5 mg/m^3

Studies of reflex response to subthreshold odor-perception toluol concentrations were made with the cooperation of 4 test persons highly sensitive to this compound. The biocurrents were recorded from the frontal-central and temporal-occipital regions. Toluol concentrations of 1 mg/m^3 produced in all the subjects statistically reliable rise in the biocurrents included in the left temporal-occipital brain regions. The reliability of such increases rose toward the 5-6th minutes after the start of gas-air inhalation and persisted to the end of the recovery period (Table 5).

Table 5

STATISTICALLY RELIABILITY OF BRAIN ELECTROPOTENTIAL CHANGES EFFECTED BY THE INHALATION OF AIR CONTAINING 1 MG/M^3 OF TOLUOL

Initials of observed	Recovery period			Recovery period		
	Minutes			Minutes		
	1–2	3–4	5–6	1–2	3–4	5–6
V.S.	b	o	o	c	a	o
N.V.	b	o	c	b	c	c
P.N.	o	c	b	c	b	a
S.L.	a	a	a	a	a	c

Note: Degree of statistics reliability: a – 95%; b – 99%; c – 99.9%; o – not reliable

No statistically reliable response was observed at 1.0 and 0.6 mg/m^3 concentrations of toluol.

Data on changes of cortical electropotentials in test person N at different toluol concentrations are presented graphically in Fig. 2.

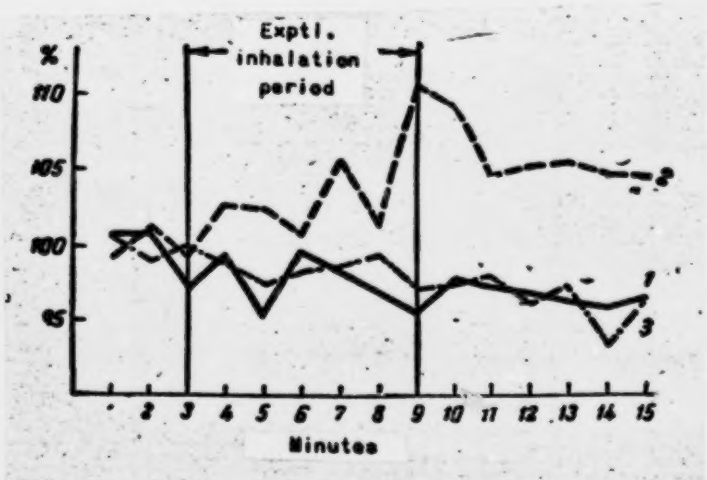

Fig. 2. Brain electropotential changes in Female N effected by the inhalation of air containing different toluol vapor concentrations

1 – Clean air; 2 – 1 mg/m^3 of toluol; 3 – 0.6 mg/m^3 toluol vapor

Four test persons cooperated in studies conducted for reflex effects manifested by different xylol concentrations. Biocurrents were recorded from the temporal-occipital regions. Xylol concentrations of 0.32 mg/m^3 elicited statistically reliable drops in the electropotentials in both temporal-occipital hemispheres in all test persons, the left hemisphere showing the most pronounced effect. Degree of reliability appeared at the 5-6th minute after initiation of gas-air inhalation and lasted to the end of the recovery period (Table 6).

Table 6

STATISTICAL RELIABILITY OF BRAIN ELECTROPOTENTIAL CHANGES EFFECTED BY THE INHALATION OF AIR CONTAINING 0.32 MG/M^3 OF XYLOL

Initials of test persons	Gas air mixture			Recovery period		
	Minutes			Minutes		
	1–2	3–4	5–6	1–2	3–4	5–6
A.K.	o	a	o	b	a	o
N.V.	o	b	c	b	c	c
P.N.	a	o	b	c	b	c
S.L.	b	a	a	b	b	b

Note: Statistical reliability: a – 95%; b – 99%; c – 99.9%
o – Not reliable

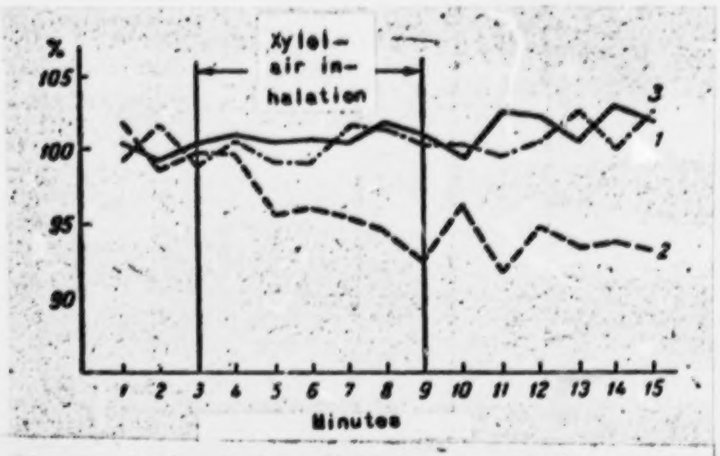

Fig. 3. Brain electropotential changes during exptl. inhalation of different xylol concentrations by male tester N
1 - Clean air; 2 - 0.32 mg/m^3; 3 - 0.21 mg/m^3

Results obtained in this study are listed in Table 7 for purposes of comparison and evaluation of effects of benzene, toluol, and xylol microconcentrations on the electrical activity of the cerebral cortex.

On this basis the present author proposes the following conclusions:

Table 7

STUDIES AND RESULTS OF ODOR PERCEPTION THRESHOLDS AND OF REFLEX EFFECTS OF BENZENE, TOLUOL, AND XYLOL CONCENTRATIONS ON ELECTRICAL BRAIN ACTIVITY

Substance	Odor Perception		Reflex effects on brain biocurrents	
	Concentration in mg/m^3			
	Threshold	Subthreshold	Threshold	Subthreshold
Benzene	2,8	2,5	2,0	1,5
Toluol	1,5	1,27	1,0	0,6
Xylol	0,6	0,41	0,32	0,21

On this basis the present author proposes the following conclusions:

1. Odor perception thresholds decreased with increase in the number of methyl groups in the benzene ring.

2. Concentrations of odor perception thresholds used as indexes in evaluating effects on cerebral electrical activity decreased benzene, toluol, xylol in the following order:

3. Benzene and toluol enhanced the electrical potentials, while xylol had the opposite effect, causing a decrease in the electrical activity of the cerebral cortex. Return to normal electrical brain activity in toluol and xylol poisoning proceeded slowly.

4. 1.5 mg/m^3 of benzene, 0.6 mg/m^3 of toluol, and 0.2 mg/m^3 of xylol are subthreshold concentrations as shown by results of electrical potential brain tests. These concentrations are recommended as maximal permissible single-exposure concentrations for atmospheric air. This recommendation was checked and approved by the Section of the Sanitary Protection of Atmospheric Air of the All-Union Sanitary Study Committee.

EXPERIMENTAL DATA PROPOSED AS A BASIS FOR THE DETERMINATION OF MAXIMAL ALLOWABLE AMMONIA CONCENTRATION IN ATMOSPHERIC AIR

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Ammonia is the simplest nitrogen-hydrogen compound; it is a colorless gas with a sharp pungent odor and biting taste. It liquifies under pressure or cooling. It is easily soluble in ether, alcohol and oils. It dissolves easily in large quantities in water, forming ammonium hydroxide. It reacts actively with acids and other compounds. Ammonia is made most commonly from nitrogen and hydrogen (I. D. Fotonich, 1956; S. A. Beskov, 1962). It is a valuable nitrogen-containing liquid fertilizer, and is also the basic raw material for the production of nitric acid and ammoniacal fertilizers. Large quantities of it are used for the preparation of ammonium hydroxide and ammonium chloride. Ammonia is widely used in the refrigeration technology, in soda production and in nitrate treatment of steel products.

Nitrate combines are the principal dischargers of ammonia into the air; other sources include nitric acid and ammonium salt refrigeration plants, coke oven gas and tanning plants as well as animal product processors. Nitrate combines and coking plants for ferrous metallurgy are most responsible for this. Considerable atmospheric air pollution with ammonia comes from the soil and open waterways. Literature survey disclosed scanty data regarding ammonia in the air surrounding such production plants and residential areas.

V. A. Ryazanov (1961) found ammonia concentration between 20.1-57 mg/m^3 on the premises of one of the largest plants synthesizing ammonia and 0.1-0.2 mg/m^3 at a radius of 1 km from a large ammonia plant. The average daily concentration at different points on the premises varied between 0.216-3.309 mg/m^3. At 2 km from the chemical plant, the concentration of ammonia did not exceed 0.004-0.005 mg/m^3 in the city air.

A study was conducted of the degree of atmospheric air pollution with ammonia around the Novolipets Metallurgical Factory, the largest source of ammonia discharge into the atmospheric air. The factory grounds house coking and nitrate plants. Ammonia is found in the carbon dioxide gas and in the waste water of coke and chemical plants. Ammonia is found in the air as a result of coke gas loss from leaks in gas conduits, scrubbers, exhaust gases, etc. Ammonia gas also comes from open ammonia storage and measuring tanks and from waste water storage reservoirs.

Ammonia is synthesized in nitrate production from coke oven gas, discharged by the coke chemistry industry. Regardless of the closed technological system the gas escaped into the air during repairs, tank drainage, transfer, etc.

Studies of the air around the Novolipets Metallurgical Combine were conducted during September-October 1963 and May-June 1964 and 1965, in connection with a study of the nitrate industry and the synthesis of liquid ammonia. Four hundred air samples were collected on the leeward side of the source at different distances and at 1.5 meters above the ground.

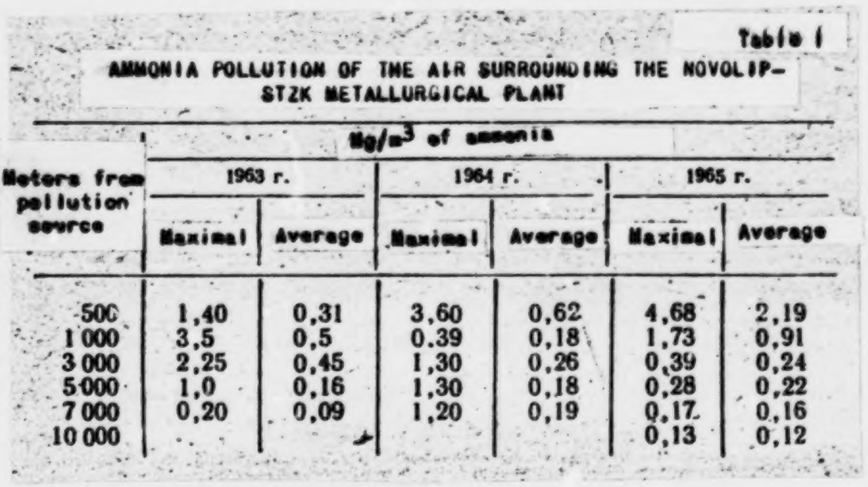

Table 1

AMMONIA POLLUTION OF THE AIR SURROUNDING THE NOVOLIPSTZK METALLURGICAL PLANT

Meters from pollution source	Mg/m^3 of ammonia					
	1963 r.		1964 r.		1965 r.	
	Maximal	Average	Maximal	Average	Maximal	Average
500	1,40	0,31	3,60	0,62	4,68	2,19
1 000	3,5	0,5	0.39	0,18	1,73	0,91
3 000	2,25	0,45	1,30	0,26	0,39	0,24
5 000	1,0	0,16	1,30	0,18	0,28	0,22
7 000	0,20	0,09	1,20	0,19	0,17	0,16
10 000					0,13	0,12

Results of the studies shown in Table 1 indicate that the greatest ammonia concentrations were found at 500 and 1000 m from the original source. These concentrations rose to higher values when the plants were in operation.

Some authors studied air pollution around less prolific points of ammonia discharge. Thus, Z. D. Markova (1941) found, in residential areas of Rostov-on-Don, ammonia in 0.02-0.05 mg/m^3 concentrations, whereas in the city parks the concentration did not exceed 0.02 mg/m^3. V. A. Kononova and V. B. Aksenova (1963) found ammonia in 0.015-0.057 mg/m^3 in the air around stock farms in a zone up to 150 m. According to American authors, ammonia concentrations varied from 0.0058-0.0143 mg/m^3 in clean air areas of Chicago (cited by V. A. Ryazanov, 1961. The concentration of ammonia in clean air, according to Stoklaz, varied between 0.02-0.04 mg/m^3, which according to Ryazanov is too high. Thus, the available data suggest that, depending on the pollutant source, ammonia concentrations in the air varied from 0.015-0.057 mg/m^3, to areas where special pollutant sources are nonexistent where the concentration varied between 0.003-0.005 mg/m^3.

Ammonia is an irritant gas, affecting primarily the mucous membranes of the respiratory tract and the central nervous system (CNS). Such action is a consequence of ammonia's high solubility in the moisture coated surfaces of the mucous membranes and its easy penetration into the blood stream. Cases of acute ammonia poisoning have been described which occurred as a result of storage tanks bursting or of ammonium hydroxide spillage in everyday use (R. N. Vol'fovskaya and G. N. Davydova, 1945; E. I. Lyublina, 1948; V. K. Trutnev and N. V. Velikorussova, 1955; K. V. Yegorov, 1958, and others). R. N. Vol'fovskaya and G. N. Davydova (1945), V. K. Trutnev, Lehmann (1886-1899), Horvath (1926-1929) and others classed ammonia as a respiratory poison with invasive necrotic effects, and R. N. Vol'fovskaya and G. N. Davydova were of the opinion that ammonia also had resorptive properties. Damage to the nervous system resulted in loss of consciousness and high excitation verging on incoherence. I. P. Pavlov (1896) noted that ammonia administered to animals in 50 mg/kg doses induced drowsiness and ataxia, and at 2½ times that dose, tremors and death. Studies conducted by V. V. Pravdich-Neminskiy (1958), Ye. A. Vladimirova (1938), E. E. Kosyakov (1962), N. B. Kozlov (1962), E. E. Martinson (1962) and others confirmed Pavlov's observations.

Investigations of Recine (1956) demonstrated that ammonia poisoning adversely affected the blood clotting process through its destructive effect on thrombin and residual nitrogen balance in the blood increases. Increased ammonia concentration in the blood and tissues produced physicochemical and structural changes in tissue proteins, disrupted ion exchange and acetylcholine metabolism, and lowering tissue respiration (N. B. Kozlov, 1963). Chronic effects of low gaseous ammonia concentrations elicited catarrhal symptoms in the upper respiratory tract, conjunctivitis and a lowering of body resistance to infectious diseases. City sewerage workers who have been exposed to low ammonia and hydrogen sulfide concentrations in the air were victims of chronic hypertrophic catarrh, leading to atrophy (M.D. Ayzenberg, 1927; T. V. Ass, V. V. Vol, et al., 1926). Abadi, Trakhta, and Trusso (cited by R. M. Zhmudskaya, 1933) found conjunctivitis and destruction of palatal membranes in workers exposed for a long time to small ammonia concentrations.

According to Henderson and Haggard (1930) the threshold ammonia concentration perceived by man was 37 mg/m^3. The threshold concentration of ammonia capable of eliciting reflex effects in man, according to T. M. Alpatov (1964) is 22 mg/m^3. The maximum allowable ammonia concentration in chronic exposure according to Leman (1896), Silverman, Whittenberger, Muller (1949) is 69 mg/m^3. The maximal allowable ammonia concentration in industrial premises adopted in 1930 by the Moscow Institute of Labor Protection was 20 mg/m^3. The number of sources which contributed ammonia as an atmospheric air pollutant is great, yet few studies have been made to determine the significance of this fact, and available data were judged to be of contradictory character. The object of the present investigation was to determine experimentally maximum allowable single average-daily tolerable concentrations of ammonia in atmospheric air. Experiments were conducted with a 25% solution of the gas.

Ammonia concentration in the air was determined colorimetrically by the Nessler method. The sensitivity of this method is 0.3 micrograms of ammonia per sample. The maximum single ammonia concentration in the air was determined by the odor perception threshold of the gas as recommended by The Committee on Clean Air Protection (1957). Twenty-two healthy persons, aged 17 to 48 years, participated, and 432 studies over a concentration range from 0.4 to 5,0 mg/m^3 were conducted.

Table 2

THRESHOLD OF AMMONIA ODOR PERCEPTION

Number of test persons	No. of determinations	Ammonia concn. in mg/m^3	
		Minimal perceptible	Maximal non-perceptible
2	36	1,95	1,0
7	138	0,98	0,75
9	200	0,70	0,55
4	58	0,50	0,45

The threshold of ammonia perception in the most sensitive test persons was 0.5 mg/m^3. Results of numerous observations indicated that ammonia elicited reflex response in threshold odor perception concentrations, which originated in the optical analyzer and in the cerebral cortex response was taken as the index in the present study. Effects of ammonia on the human body were determined with the aid of an adaptometer ADM in cooperation of three test persons whose threshold of ammonia odor perception was previously determined. Tests were conducted with 0.65 and 0.51 mg/m^3 concentrations for one test person and 0.51, 0.45 and 0.32 mg/m^3 for the other two, based on the differences in the threshold detection levels in the test persons. Effects of each concentration were observed at least three times. Obtained data indicated that, ammonia in 0.45 mg/m^3 concentration lowered eye sensitivity to light in two persons (D. N. and L.N.)

while 0.32 mg/m^3 concentration was inactive (Fig. 1). In the third test person (Sh. L.), the threshold odor perception concentration which affected eye sensitivity to light was 0.65 mg/m^3 and the subthreshold concentration was 0.5 mg/m^3.

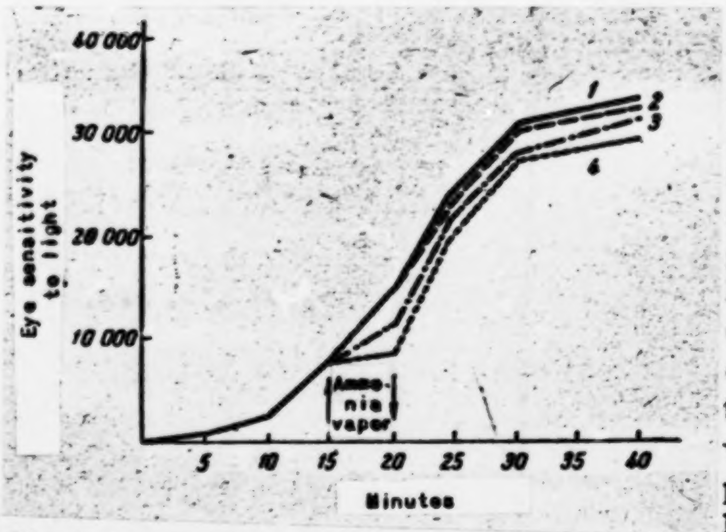

Fig. 1. Eye sensitivity to light changes in female D.N. effected by inhalation of air containing different concns. of ammonia. 1 - Clean air; 2 - Ammonia 0.32 mg/m^3; 3 - Ammonia 0.45 mg/m^3; 4 - Ammonia 0.5 mg/m^3

The threshold ammonia conconcentration which affected electrical cerebral cortical activity was determined by continuous recording of the alpha-rhythm evoked by flashing of light. The work was done at the Department of Community Hygiene of the Central Postgraduate Medical Institute with cooperation of 5 test persons aged 18 to 24 whose concentrations of threshold perception of ammonia odor had been previously determined. Changes in biopotentials were registered on an 8-lead "Kayzer" electroencephalograph. Analysis of overall cerebral-cortical activity was made by using a multilead Balashev (1964) integrator. Biocurrents were registered by the bipolar method. Periodic light pulses were flashed from a photostimulator at a frequency of 8 flashes per second and intensity of 0.1, 0.2, and 0.6 i. Three tests were made with ammonia concentration and additional 2 or 3 control runs with clean air. Total bioelectrical activity during the entire period of optical stimulation was calculated by means of an integrator. Results were recorded in percentages of average activity level computed for the first 3 minutes, which assigned the value of 100%.

In three test persons whose odor perception threshold was 0.55 mg/m^3, ammonia concentrations of 0.35 mg/m^3 affected the cerebral-cortical electrical activity and in two of the test persons (Kh. V. and K.V.) a drop in total bioelectric activity occurred in the first minute, and in test person D. M., bioelectrical activity was enhanced. Concentrations of 0.22 mg/m^3 produced no changes (Fig. 2) in any of the 3 test persons.

In two subjects (D. N. and I. N.) with thresholds of 0.76 mg/m^3, the activating concentration was 0.44 mg/m^3 and the subthreshold concentration was 0.32 mg/m^3. Clear-cut ammonia effects appeared in the fourth minute followed by a weakening between the 5-6th minutes. A more pronounced ammonia effect on the organism during the first exposure minute can be also seen while determining ammonia odor perception threshold and during the study of eye sensitivity to light.

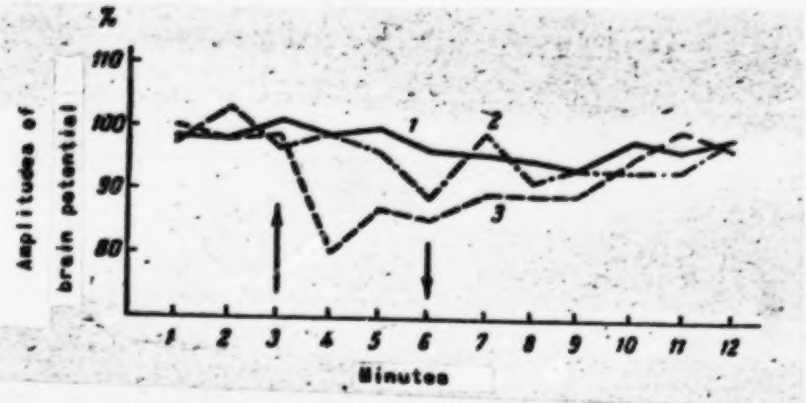

Fig. 2. Brain potential amplitudes of female K. V. in the course of inhaling air containing different concentrations of ammonia vapor.
1 - Clean air; 2 - Ammonia 0.22 mg/m^3; 3 - Ammonia 0.35 mg/m^3
Zone between arrows period of air-ammonia vapor inhalation

Thresholds of ammonia reflex activity are presented in Table 3.

Table 3

THRESHOLD REFLEX EFFECT OF AMMONIA

Index investigated	Concentration in mg/m^3	
	Threshold	Subthreshold
Odor perception	0,50	0,45
Changes in eye sensitivity to light	0,45	0,32
Changes in electrical brain activity	0,35	0,22

Accordingly, the 0.2 mg/m^3 concentration which was designated as the subthreshold on the basis of the most sensitive test results is here recommended as the maximal allowable single concentration in atmospheric air.

Determination of average daily allowable concentration of ammonia in atmospheric air was studied by effects on animals under conditions of chronic experiments. White rats were exposed daily for 24 hours to the inhalation of ammonia air mixtures in different concentrations during an uninterrupted period of 84 days. Rats of Group I inhaled air containing ammonia in 20 ± 0.1 mg/m^3 concentration which is the maximal allowable concentration for air in industrial areas; Group II-- 2.0 ± 0.61 mg/m^3; and Group III-- 0.2 ±.0072 mg/m^3 which is identical with the concentration determined as the maximum allowable single concentration for the atmospheric air; Group IV, controls. Sixty white rats, weighing 105-150 were selected for the chronic inhalation experiment. Rats were housed in 100 liter chambers. Clear air containing gaseous ammonia in known concentrations was continuously run into the chamber at the rate of 28-30 li/min. Air samples for analysis were taken daily.

In the course of the continuous chronic experiment the general condition of the rats, their weight, latent reflex time, porphyrin metabolism, and urinary ammonia content were recorded. Blood serum cholinesterase, redox activity, luminescent leucocyte content, hemoglobin, erythrocytes and nucleic acids were determined. At the end of the inhalation exposure and the recovery period, some of the rats were sacrificed for post mortem study.

Experimental results were evaluated statistically as described in some of the preceding reports:

During the experimental period rats of all groups were healthy, active, and maintained the normal rate of gain in body weight.

Data obtained on latent reflex-activity time served as the basis for evaluating functional state of the CNS effects produced by the chronic ammonia inhalation experiment, as suggested by A. A. Minayev (1965), A. P. Fomin (1965), and using the S. I. Gorshkov "chronoreflexogenometer". Determinations were made in sigmas of the time which elapsed between an electric motor stimulus and reflex response to it.

Results of the study are shown in Table 4 and graphically in Fig. 3.

Table 4

CHANGES IN LATENT REFLEX TIME OF RATS

Periods	Group			
	I (20 мг/м^3)	II (2 мг/м^3)	III (0,2 мг/м^3)	IV Control
Before inhalation	71,4(o)	69,2(o)	70,4(o)	71,3
1st inhalation month	57,6(c)	66,4(o)	67,8(o)	67,0
2d inhalation month	66,3(o)	68,0(o)	67,6(o)	68,3
3d inhalation month	68,3(o)	68,4(o)	68,4(o)	68,1
Recovery period	68,0(o)	68,8(c)	68,0(o)	68,2

Note: Statistical reliability: c – 99.9%; o – not reliable

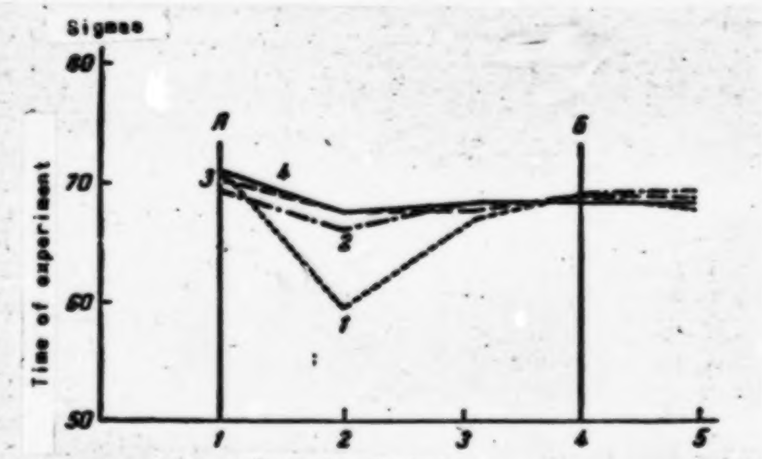

Fig. 3. Changes in latent period of reflex response in rats effected by the inhalation of air containing different ammonia concentrations
1 – Group I (20 mg/m^3); 2 – Group II (2 mg/m^3); 3 – Group III (0.02 mg/m^3); 4 – Group IV; AB – Period of experimental inhalation

The time of latent motor reflex response diminished in rats of Group I in the first month of the inhalation exposure to 20 mg/m^3, ammonia concentration.

Blood cholinesterase activity was determined by the Fleischer-Pauns method as modified by N. N. Pushkina and N. V. Klimkina (N. N. Pushkina, 1963). Diminished cholinesterase activity was observed in rats of Group I. No changes appeared in rats of the other group judged by the graphic records in Fig. 4

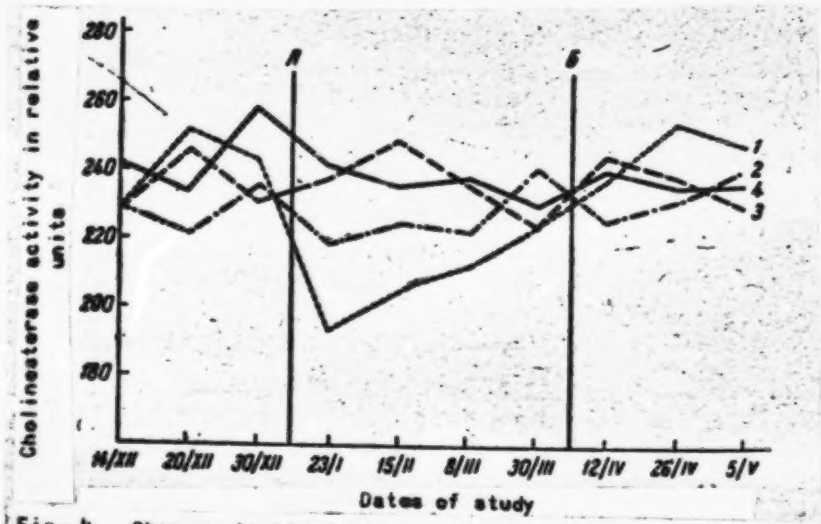

Fig. 4. Changes in blood cholinesterase activity or rats effected by inhalation of air containing ammonia (Notations same as in Fig. 3)

Redox function of the blood serum was determined by a modified Tumberg method (Yu. L. Anin, 1964) which is based on the methylene blue color change in blood serum heated over a boiling water bath and recording the time required for complete color disappearance. Complete disappearance in the instances of rats of Groups I and II (Table 5, Fig. 5) proceeded at a slower rate pointing to a drop in redox activity in the animal body.

Table 5

CHANGES IN THE REDOX FUNCTION IN BLOOD SERUM OF RATS IN MINUTES

Periods	Groups			
	I (20 мг/м^3)	II (2 мг/м^3)	III (0,2 мг/м^3)	IV (Control of IV Group)
Prior to inhalation	10,53(o)	10,93(o)	10,46(o)	10,66
Inhalation period	19,10(c)	15,0(c)	12,20(o)	12,05
Recovery period	12,66(o)	12,73(o)	12,63(o)	12,66

Note - Statistical reliability - c - 99.9%; o - Statistically not reliable

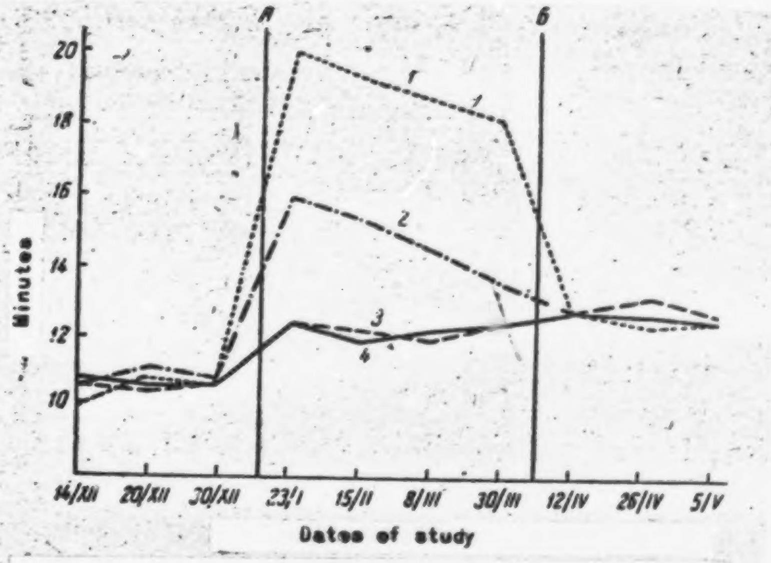

Fig. 5. Effect of ammonia on the redox function of rats' blood serum
Notations same as in Fig. 3 .

Luminescence under the microscope has been used as an index in determining early qualitative changes in the morphology of white blood elements. The method is based on the capacity of nucleoproteins of degenerating cells to combine with acridine orange more than with the nucleoproteins of newly generated cells (M. N. Meysel', V. A. Sondak, 1956). This phenomenon was not observed in the present study. No changes were noted in erythrocyte counts, hemoglobin, concentration, and in total nucleic acid content.

Yu. K. Smirnov (1953) showed that stimulation of the nervous system was followed by an increase in porphyrin metabolism and a retarded return to normal. Gusev (1960) first included porphyrin metabolism studies as a guiding index in evaluating the presence of lead in atmospheric air. Other scientists, G. I. Solomin (1961), Li Shen (1961), V. I. Filatova (1961), B. M. Mukhitov (1963) and others confirmed the high sensitivity of this method. The rate of coproporphyrin elimination via the urine was determined spectrophotometrically. During the inhalation exposure, only rats of Group I showed coproporphyrin changes (Fig. 6).

Results of chronic daily 24-hour exposure of white rats to ammonia-air inhalation in concentrations of 20 mg/m^3 shortened reflex time, lowered blood cholinesterase activity and the redox function, and also increased the rates of coproporphyrin and ammonia eliminations via the urine.

In 2 mg/m^3 concentrations ammonia lowered the redox function of the blood serum. In 0.2 mg/m^3 concentration in the air ammonia elicited no physiological change in the experimental rats. Pathohistological studies disclosed no significant changes in the internal organs and in the CNS in the experimental rats.

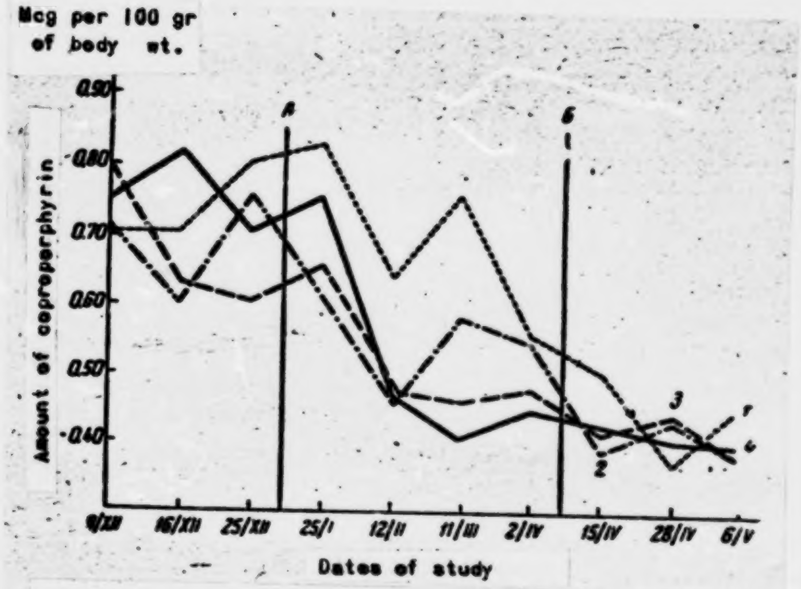

Fig. 6. Effect of air-ammonia inhalation of rats' coproporphyrin metabolism
Notations same as in Figure 3.

CONCLUSIONS

1. Many industries, especially metallurgical plants, having coke oven gas and nitrate operations on the premises, constituted the chief source of atmospheric air pollution with ammonia.

2. Subthreshold ammonia concentration which had no effect on the cerebral cortex biopotentials is at the 0.2 mg/m^3 level, which can be regarded as its maximal allowable single concentration in atmospheric air.

3. Under conditions of chronic inhalation exposure such a concentration proved toxicologically inactive. On this basis such ammonia concentration can be recommended as its maximal allowable limit in atmospheric air.

CHRONIC EFFECTS ON THE ORGANISM OF SMALL CONCENTRATIONS OF ACRYLALDEHYDE IN AIR

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Acrylaldehyde (AAD)* is produced industrially by oxidizing propylene over CuO or a vapor-phase condensation of acetylaldehyde with formaldehyde in the presence of Zi_3PO_4. Much AAD is consumed in producing glutaric aldehyde and methionine. AAD is an important product of glycerine synthesis. It gets into the atmosphere when glycerine undergoes industrial treatment at high temperatures (130-180° C): in manufacturing linoleum and oilskin, in the core sections of foundries, and in manufacturing insulation in the electrical engineering industry. It has been shown in recent years that AAD is a constituent of automobile exhausts.

V. X. Yas'kova has found in the outside and indoor air around the Leningrad Grease Combine high concentrations of AAD which may have been due to the use of a nonspecific analytical method. M. M. Plotnikova (1960) made quantitative studies of AAD in atmospheric air around chemical and oil plants. At a distance of 100 m from the latter the maximum concentrations were 2 mg/m^3, and in all samples the amount of AAD exceeded the maximum allowable 0.3 mg/m^3, while at a distance of 1000 m in the maxima were 0.65 mg/m^3. At a distance of 200 m from the drying-oil shop of a chemical plant the maximum concentration was 20 mg/m^3; at 1500 m, 0.3 mg/m^3. AAD acts as a strong mucous membrane and as a general nonspecific toxin (N. V. Lazarev, 1954). The maximum allowable AAD concentration for air of indoor working premises was set at 0.002 mg/li. Plotnikova used the threshold of odor perception technic in determining the allowable maximal single exposure concentration of 0.8 mg/m^3 of acrylaldehyde in the air. The threshold of optical chronaxy concentration was determined at 1.75 mg/m^3, and for eye sensitivity to light it was 0.6 mg/m^3. The maximal allowable single concentration of acrylaldehyde in the air was found as 0.3 mg/m^3. The maximal allowable 24-hour single concentration was determined by a process of computation without experimental verification, and was set at 0.1 mg/m^3.

Effect of low acrylaldehyde concentrations in the air on the living organism was studied by the procedure recommended by the Committee for the Sanitary Protection of Atmospheric Air, by exposing 40 white rats to daily 24-hour continuous inhalation of AD over a period of 60 days. The rats were divided into 4 equal numbered groups; three of the groups were housed in special inhalation chambers; animals of group 4 served as controls. Acrylaldehyde concentrations in the chambers were determined by the Plotnikova modification of D. P. Senderikkhina's tryptophan method. In reacting with tryptophan AAD formed a stable violet dye. The method is accurate to 0.002 mg in 2 ml of solution. In the present study specificity of the method was not of importance (M. V. Alekseyeva, 1962).

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*Cf. the article of M. M. Plotnikova in the collection Maximum Tolerable Concentrations of Atmospheric Pollutants, edited by Prof. V. A. Ryazanov, 1960, No. 4.

The AAD concentrations in the chambers housing Groups I, II, and III were correspondingly as follows in mg/m^3: 1.52 ±05 with fluctuations from 1 to 2 mg/m^3; 0.51± 0.2 with fluctuations from 0.22 to 0.77; and 0.15 ±0.01 with fluctuations from 0.1 to 0.3.

The following indexes were used in the study: changes in weight and behavior, in conditioned-reflex activity; changes in whole-blood cholinesterase activity, in rate of coproporphyrin elimination via the urine, in the percentage of luminescent leucocytes, and pathological changes in the organs and tissues of post mortem rats which died during the inhalation tests, or were sacrificed at the end of the experiment. At the end of the first week rats of Group 1 (1.52 mg/m^3) appeared sickly, sluggish and apathetic, and the fur became lusterless; they ate poorly. The condition of the rats became progressively worse, and inhalation was discontinued on the 24th day. Before this, 5 rats (whose central nervous system was studied) died, and 2 died subsequently. By contrast, the rats in the other three groups exhibited no changes in their general behavior.

Table 1 summarizes changes in the weekly weights of each group.

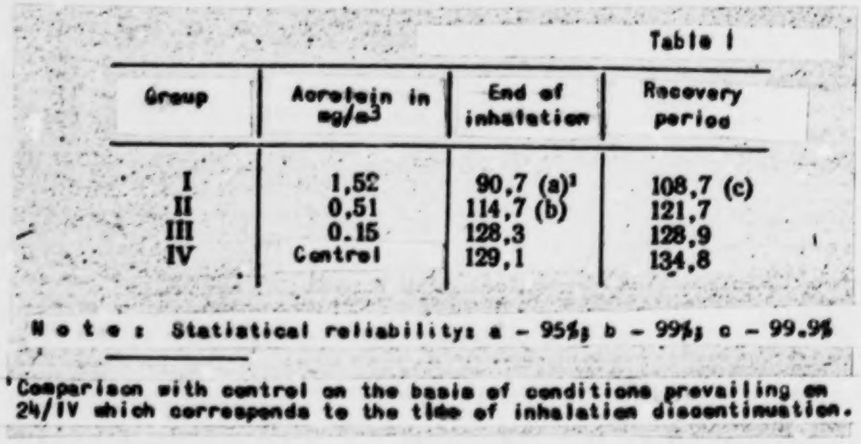

Table 1

Group	Acrolein in mg/m^3	End of inhalation	Recovery period
I	1,52	90,7 (a)[1]	108,7 (c)
II	0,51	114,7 (b)	121,7
III	0.15	128,3	128,9
IV	Control	129,1	134,8

Note: Statistical reliability: a – 95%; b – 99%; c – 99.9%

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[1] Comparison with control on the basis of conditions prevailing on 24/IV which corresponds to the tide of inhalation discontinuation.

Rats of Group 1 lost 14% of their initial weight in the course of the abbreviated inhalation exposure; loss in body weight continued after exposure to experimental inhalation was discontinued. By the end of the first week of the recuperation period the weight loss was 25%, and only toward the end of the 5th week of the recovery did the average weight in this group reach the initial level and continued to rise beyond it. Rats of Group II also exhibited a statistically reliable weight loss, whereas rats of Group III showed no statistically significant changes in their body weight.

Yu. V. Novikov, N. F. Izmerov, M. I. Gusev, and K. N. Chellikanov; Ya. G. Dvoksin, V. N. Kursanov, and others applied the conditioned reflex method to determine the maximum allowable concentrations of chemicals in atmospheric air. Their studies showed that changes in cerebral-cortex functions appeared very rapidly. Early symptoms of impairment in the higher nervous activity appear in the form of phase states, differential disinhibition, falling out of certain reflexes, and finally, inclusion of all stereotypical functions. Severe central nervous damage caused natural conditioned optical and odor and taste perception reflexes to disappear. The restoration period in such cases may last a month or longer.

The chief symptom or indication of changes in the higher nervous activity induced by toxic agents is constant relaxation of the stimulatory and inhibitory processes, with subsequent development of protective inhibition. The high sensitivity of the conditioned-reflex method is indicated by the fact that it was used in determining the activity threshold for benzene, lead, and mercury considerably below those established by previous chronic-inhalation experiments based on the evaluation indexes. However, the old method of studying conditioned reflexes in the Kotlyarev chambers is elaborate and time-consuming. In the present study of the higher nervous activity in white rats an accelerated method was used in the form of the Ya. G. Dvoskin modification (1961). Eighteen white rats weighing 64-102 g were divided into three groups of 5 rats each and a control group of 3 rats. The stereotype was studied in the order of bell-light; light-bell.

Group I rats (1.52 mg/m^3) exhibited the most profound changes in higher nervous activity, the conditioned respiratory response falling in comparison with the controls after inhalation began (effects had been noted with light and sound stimuli). Plase states then appeared. The conditioned reflex activity (CRA) of one of Group I rats is shown graphically in Fig. 1.

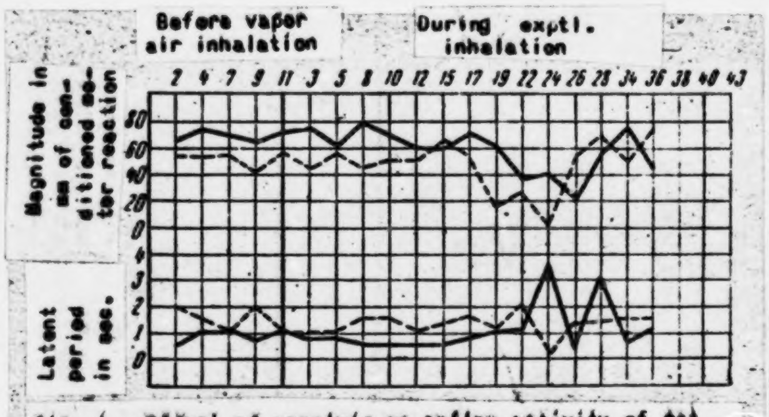

Fig. 1. Effect of acrolein on reflex activity of rat No. 12 subjected to the inhalation of air containing 1.52 mg/m^3 of the vapor.

Changes in CRA also appeared in rats of Group II (0.51 mg/m^3), but in a less pronounced form (Fig. 2).

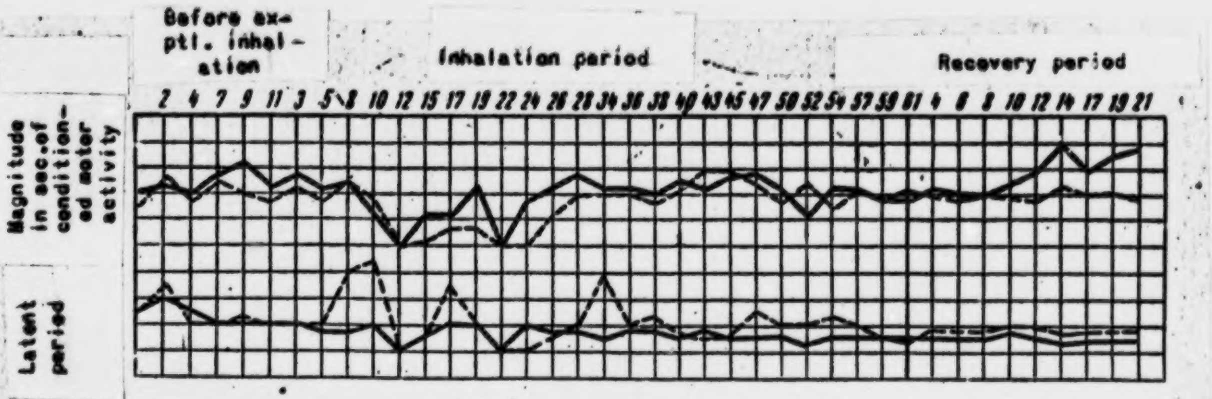

Fig. 2. 0.51 mg/m^3 acrolein effect on conditioned reflex activity of rat No. 19.

No CRA changes had been observed in rats of Group III (0.15 mg/m^3) and IV (Control), which is clearly shown in Figs. 3 and 4: throughout the inhalation exposure period these rats maintained the proper strength correlation with weak and strong stimuli, as well as in the oatent period. As previously mentioned, the Group I rats died as a result of AAD inhalation and it was impossible to study their behavior during the recovery period. On the 10th day of discontinued inhalation the cerebral-cortex activity of rats in Group II returned to normal.

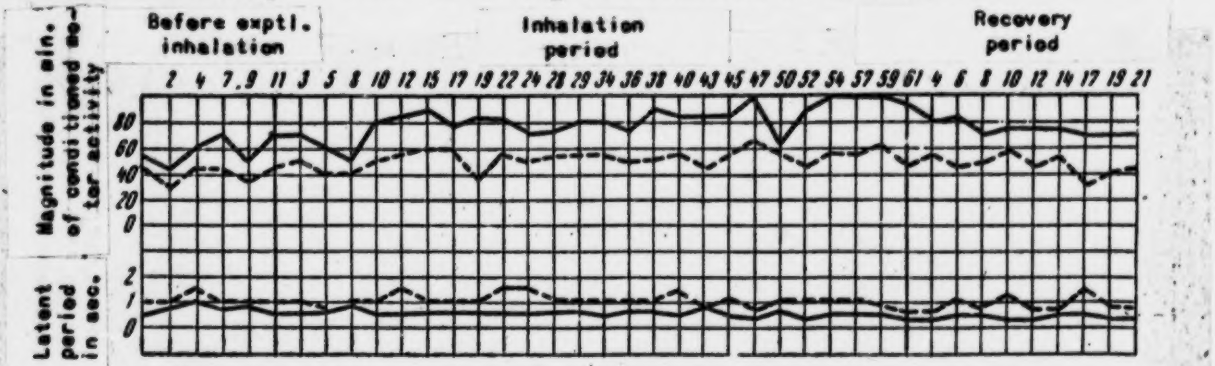

Fig. 3. 0.15 mg/m^3 of acrolein on conditioned reflex activity of rat No. 8.

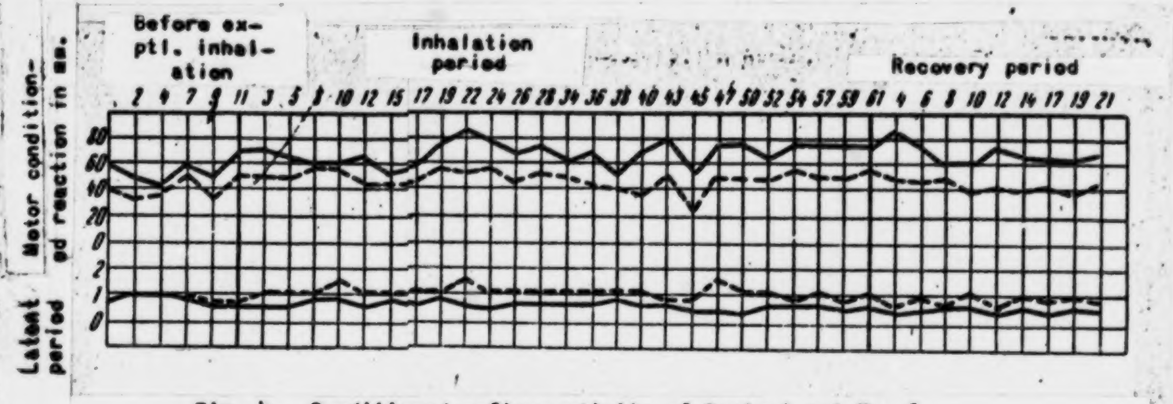

Fig. 4. Conditioned reflex activity of Control rat No. 5.

It is generally believed that changes in whole-blood cholinesterase activity reflected disturbance in the functional state of the nervous system (Ye. B. Babskiy and A. A. Kirillov, 1938; D. Ye. Al'pern, 1958) and shifts in the functional state of the organism as a whole. This index has been widely used in determining maximal allowable concentration of chemical substances in the air and has proved sensitive in a series of studies conducted under the direction of V. A. Ryazanov by G. I. Solomin (1961); D. G. Odoshashvili (1962), Li Shen (1961), R. U. Ubaydullayev (1962), P. G. Tkachev (1963), V. A. Chizhikov (1963), V. I. Filatov (1963), and others; in fact, the new index limits were 10 to 20 times below the old allowable maximal concentrations for industrial premises.

Cholinesterase activity was determined by using Pokrovskiy's method (1953) as modified by A. P. Martynova (1957). The method is based on the rate of acetylcholine hydrolysis as indicated by the rate of color change due to change in the pH factor. Cholinesterase activity was determined weekly in 5 rats of each group. Results are shown in Table 2. (Table 2 is shown on page 81).

Hydrolysis time in the control group was 38.7-40.3 min; a statistically reliable rise in the time to 61 min manifested itself in rats of Group I on the 15th day of exposure to inhalation. Two days after the experiment was terminated on the 24th day, cholinesterase activity was still below normal. The initial level was not reached until the second day following the sixty days of the regular inhalation period.

Statistically reliable changes in rats of Group II cholinesterase activity first appeared on the 34th day of inhalation and reached a maximum on the 41st day. Hydrolysis time began to drop even before the end of the inhalation period and returned to normal on the 10th day of the recovery period. No change in whole-blood cholinesterase activity was observed in rats of Group III or control rats at any time.

The basic mechanism of the effect of toxic substances on porphyrin-metabolism has not been adequately investigated. It is widely believed, however, that it suppressed the enzyme systems and affected cellular metabolism in the nervous system, liver, bone marrow, etc. (A. M. Chernyy, R. B. Mogilevskaya, and others).

The rate of coproporphyrin elimination via the urine is lowered by some toxic substances, such as carbon monoxide, dynel, styrene, dimethylformamide, and increased in the presence of other substances, such as lead, pentane, toluylene-diisocyanate.

Table 2

Minutes of acetyl-cholin hydrolysis in white rats.

Group	Concn. at end of inhalation	Before inhalation					Inhalation period				Recovery period	
		18/III	22/III	8/IV	15/IV	24/IV	6/V	13/V	20/V	28/V	11/VI	19/VI
I	1,52	37,4	38,4	39,3	61,0 (c)	56,0¹ (b)	—	44,0	—	40,0	40,5	39,7
II	0,51	41,4	40,6	40,8	41,5	40,8	47,4 (a)	52,0 (c)	48,4 (b)	44,7 (a)	38,5	39,5
III	0.15	40,4	39,6	40,4	40,5	40,0	40,0	41,2	39,3	39,9	40,0	39,5
IV	Control	39,1	39,0	38,7	40,3	39,2	39,2	39,4	39,8	39,9	39,9	39,1

Note: Reliability Degrees: a - 95%; b - 99%; c - 99.9%

¹Inhalation discontinued

Changes in porphyrin metabolism proved to be a useful and sensitive index; it was widely employed by M. I. Gusev, V.A. Chizhikov, G. I. Solomin, D. G. Ododoshvili, and others in establishing the limit of allowable pollutant concentrations in atmospheric air. Rate of coproporphyrin elimination via the urine was determined spectrophotometrically and by computing the optical density of maximum absorption at 402-403 millimicrons. An SF-4 spectrophotometer was used. 24-hour urine of 5 rats in each group was collected once a week. Thus, 56 analyses were made, 14 from each group. Table 3 shows the amount of coproporphyrin extracted from the urine of the rats and results were recorded in micrograms per 100 g of body weight.

Table 3

24-HOUR COPROPORPHYRIN ELIMINATION WITH URINE IN TERMS OF MICROGRAMS PER 100 G OF BODY WEIGHT

Study periods from inhalation initiation	Acrolein in mg/m^3			
	Control	0.15	0.51	0.54
First [illegible] weeks	1,88	1,54	1,43 (a)	1,93
Second [illegible] weeks	1,94	1,61	1,26 (b)	0,99 (c)¹
Third [illegible] weeks	1,78	1,60	0,67 (c)	0,72 (c)
Recovery period	1,87	1,61	1,50	1,59

Note: Reliability degrees a - 95%; b - 99%; c - 99.9%

1c 24/IV inhalation discontinued

No reliable metabolic changes were noted in rats of Group III; statistically reliable drop appeared in rats of Group II during the first 3 weeks of experimental inhalation. This reduction continued to the end of the inhalation period.

Rats of Group I manifested a tendency to increased elimination rate to the end of the experimental inhalation, although this change was not statistically reliable. After inoculation was discontinued porphyrin metabolism began to drop gradually, which continued until the 6th week of the recovery period, when the curve began to level out, so that during the 8th week it coincided with the level of the control group.

Table 4

PERCENT OF LUMINESCENT LEUCOCYTES

Group	Mg/m^3 of acrolein	Before inhalation 22/III	Inhalation period						Recovery period	
			8/IV	15/IV	24/IV	9/V	19/V	20/V	11/VI	19/VI
I	1,52	3,95	21,70 (c)	5,70 (a)	Inhalation discontinued	—	18,60 (b)	3,70	—	—
II	0,51	4,40	12,40 (c)	4,55	10,3 (a)	15,80 (c)	16,4 (b)	9,50 (c)	5,40	2,70
III	0,75	5,60	1,70	4,30	7,56 (a)	9,60 (b)	5,40	3,90	4,50	4,80
IV	Control	1,31	1,03	1,03	4,80	6,00	8,30	3,80	2,50	1,00

Notes: Reliability degree: a – 95%; b – 99%; c – 99.9%

With the aid of luminescent-microscopy according to M. N. Meysel' and A. V. Gutkina (1953), M. N. Myesel' and H. A. Sondak (1954), A. D. Semenenko (1963) was able to detect after secondary stimulated luminescence, changes in blood leucocytes in white rats subjected to chronic aniline inhalation in concentrations of 0.03, 0.3, and 3 mg/m^3. Depending on the concentration of the pollutant in the air, luminescence was noted in rats of all three groups.

M. I. Gusev and K. N. Chelikanov (1963) used the leucocyte luminescence method in attempting to determine daily allowable maximal pentane concentrations in atmospheric air. A statistically reliable increase in luminescent leucocyte count was observed in their studies. The leucocyte count increase persisted through the inhalation period, but returned to normal during the recovery period.

Table 4 summarizes the results of tests made to determine, with an Ml-1 microscope, the percent content of luminescent leucocytes in the blood fluorochromed with acridine orange diluted 1:10,000. During the first week of experimental inhalation, rats of Group I (1.52 mg/m^3 exhibited a leucocyte increase. The count subsequently decreased to levels still above those of the controls. It was difficult to get blood from rats of Group I due to its rapid coagulation, which explains the gaps in leucocyte data on some days of the experiment. The increased leucocyte counts persisted for 20 days after the premature termination of the inhalation period; it returned to normal 7 days later, after which no further tests were run.

Rats of Group II (0.51 mg/m^3) also manifested a statistically reliable change in leucocyte percentage during the first week, a change which continued to the end of the experimental inhalation. After 11 days of recovery the leucocyte count began to drop and returned to normal.

Rats in Group III, which had shown no indications of being affected by AAD in any of the other tests, exhibited a statistically reliable increase in leucocyte percentage on the 24th day of inhalation. Other investigators noted similar changes. For example, A. D. Semenko (1963) produced reliable changes in the blood of rats chronically exposed to the inhalation of aniline in a concentration of 0.03 mg/m^3, while P. G. Tkachev (1963), who used different methods, detected no changes with the same concentration. This disparity in results suggests that luminescent microscopy is a highly sensitive method which can be used in determining the maximum allowable concentration of chemical substances in atmospheric air. In this connection the necessity arises to study the nature of the process by which luminescing-leucocyte count increased when toxic substances in low concentrations affected the organism.

After the chronic experiment was ended some of the animals underwent patho-anatomic study.* Histological examination of organ tissues of animals in Groups III and IV revealed no noteworthy irregularities. The first changes, at a concentration of 0.51 mg/m^3 (Group II) appeared as proliferation of the cylindrical epithelium of the bronchi with hyperproduction of mucous and excessive infiltration of the bronchial walls by eosinophiles. Rats subjected to the inhalation of air containing 1.52 mg/m^3 of AAD (Group I) exhibited acute changes of an inflammatory nature: in the respiratory organs in the form of purulent panbronchitis and bronchiolitis, and macrofocal pneumonia in one half of the rats; and in two cases in the form of fibropurulent pleurisy. In the internal organs (miocardium, liver) acute dystrophic changes were noted in the form of granular and fatty dystrophy (degeneration) with small sections of necrobiosis.

CONCLUSIONS

1. Prolonged inhalation of acrylaldehyde (AAD) vapor in 0.51 and 1.52 mg/m^3 concentrations caused weight loss, changes in conditioned-reflex activity, a reduction in cholinesterase activity, a drop in porphyrin metabolism, and an increase in the percentage of luminescent leucocytes in the blood of the experimental animals.

2. Aside from an increase in leucocytes, no changes were effected by the inhalation of 0.15 mg/m^3 of AAD under identical circumstances. The secondary luminescent method presents the opportunity to directly observe the earliest physiochemical and morphological changes in the cells. Therefore, it can be recommended for determining the average daily maximum allowable concentration of a pollutant in atmospheric air.

3. The presently established maximal average daily allowable concentration of AAD in atmospheric air (0.1 mg/m^3) is below the threshold of activity and may be retained without change.

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*Conducted by K. L. Volchenko.

HYGIENIC EVALUATION OF THE COMBINED EFFECTS OF ACETONE AND PHENOL IN ATMOSPHERIC AIR

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Experimental studies of the biological effects of atmospheric pollution found wide acceptance in the Soviet Union in determining hygienic standards for maximum allowable harmful substances concentrations in atmospheric air. At this writing, 67 such pollutants had their individual maximal fixed. However, a single industrial plant will often discharge into the air a mixture of pollutants. Industry employs highly complex combinations of compounds which can react with each other and form new, and sometimes more toxic, compounds. In addition, an inhabited district may have several different industries, each discharging into the air its type of pollutant. For this reason, the continued investigation into the effects of individual pollutants must be supplemented by studies of the effects of two or more pollutants simultaneously present in the air.

While the literature dealing with such problems is scant, such studies as have been made show that the combined effect of air pollutants a, b, c...is as a rule the sum of their individual effects: e.g., sulfur dioxide and the aerosol of sulfuric acid (K. A. Bushtuyeva, 1961) hydrogen sulfide, carbon disulfide, and dynel (Kh. Kh. Mannanova, 1964); ethylene, propylene, butylene, and amylene (M. L. Krasovitskaya, 1964); isopropylbenzene and its hydroperoxides (G. I. Solomin, 1964); and strong mineral acids (sulfuric, hydrochloric, and nitric) in concentrations of H-ions (V.P. Melekhina, 1964). The results of these experiments are included in the table of MAC of contaminants in atmospheric air which was approved at the end of 1965.

Among the promising technological processes in the world's organo-synthetic industry is the method of producing acetone and phenol simultaneously through isopropylbenzene hydroperoxide (cumene). It was developed and first introduced on an industrial scale in the Soviet Union in 1949. In connection with the increasing demand for acetone and phenol in different branches of the national economy, the cumene method, being the most economical, has found widespread use; plants employing this method can be regarded as future potential atmospheric air pollutants from the standpoints of air protection because of the imperfection of the production processes involved and other similar factors.

It has been shown by V. P. Melekhina, M. A. Pinigin (1961) and others that the air around a plant producing acetone and phenol simultaneously is polluted over extended distances by these two substances and others. Therefore, investigating the combined effect of phenol and acetone in low concentrations is of pressing theoretical and practical importance, especially in relation to determining limits of concentrations from the sanitary hygienic viewpoint.

Acetone (dimethylketone) belongs to the family of compounds which possess cumulative irritant and narcotic properties. Phenol (carbolic acid) is a nerve poison; it also has irritant and narcotic properties (N. V. Lazarev, 1951). In this study the nephelometric method was used in determining phenol and acetone in atmospheric air (M. V. Alekseyeva, B. Ye. Andronov, S. S. Gurvits, A. S. Zhitkov, 1954) and the colorimetric method for the former (V. A. Zhrustaleva, 1962). Acetone reacts with iodine in an alkali medium to form a white suspension of iodoform the intensity of which can be estimated by a standard scale. The sensitivity of the method is 0.001 mg in 4.5 ml. The method is specific, and phenol does not interfere with the determination.

The action of phenol with 4-aminoantipyrene in the presence of potassium ferrocyanide at pH= 9.3 forms a rose-colored dye. The sensitivity of this method is 0.0002 mg in 2 ml. Acetone does not interfere with the determination. The investigation began by determining odor perception concentration for the two chemicals individually, and then in combination. Eighteen test volunteers cooperated in this study.

The odor perception threshold concentration was determined by using a generally accepted method (V. A. Ryazanov, K. A. Bushtuyeva, Yu. V. Novikov, 1957). Five of the eighteen test persons whose odor perception was the most sensitive (1.1 mg/m^3) for phenol and 0.22 mg/m^3 for acetone. This was identical with results of previous investigations of Yu. G. Fel'dman (1962), B. A. Mukhitov (1963). The concentrations employed in first test are summarized in Table 1.

Table 2 presents results of odor perception threshold determinations in the five most sensitive test persons; the threshold served as an index and showed that the threshold concentration of each component of the combination was equal to 1.0. The remaining 13 testers were not sensitive to these concentrations, which is entirely natural, since they had a higher threshold of odor perception for each substance individually and in combination.

The odor perception threshold is not to be confused with the limit of physiological activity of the irritants. Often, without being accompanied by sensation, stimulants evoke definite physiological responses of a so-called subsenory nature (G. V. Gershuni, 1949). This phenomenon has been confirmed in studies with low concentrations of harmful substances in inhaled air. Investigations along this line have shown that subsenory concentrations of chemical irritants normally insensible by odor can cause changes in the functional state of different organs and systems, particularly the visual analyzer; and the threshold of such changes is considerably below that of which the subject is aware.

Table 1

ACETONE AND PHENOL CONCENTRATIONS CORRESPONDING TO THRESHOLD OF ODOR PERCEPTION OF THEIR MIXTURES

Acetone and phenol in mixture				Summary concn. of individual sub-thresholds of each component
mg/m^3		In parts of individual threshold perception		
Acetone	Phenol	Acetone	Phenol	
$\frac{1,1^*}{1,1}$	$\frac{0,022}{0,022}$	1,0	1,0	2,0
$\frac{0,72}{1,1}$	$\frac{0,011}{0,022}$	0,65	0,5	1,15
$\frac{0,55}{1,1}$	$\frac{0,011}{0,022}$	0,5	0,5	1,0
$\frac{0,55}{1,1}$	$\frac{0,008}{0,022}$	0,5	0,36	0,86
$\frac{0,36}{1,1}$	$\frac{0,011}{0,022}$	0,33	0,5	0,83

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*In the numerator - the concentration looked for; in numeraton - threshold odor concentration of individual component.

Table 2

ODOR PERCEPTION THRESHOLDS OF ACETONE PHENOL VAPOR PERCEPTION FOR PERSONS OF MOST SENSITIVE ODOR PERCEPTION

Summary concn. of threshold fractions of each component part	Experimental results	
	Perceptible	Not perceptible
2,0	5 testers	—
1,15	5 »	—
1,0	5 »	—
0,86	—	5 testers
0,83	—	5 »

Eye sensitivity to light is a fine and labile function of the visual analyzer the level of which is affected by different factors in the external environment. Particular attention should be centered on the view based on a series of studies by L. A. Orbeli (1934), A. V. Lebedinskiy (1935), K. Kh. Kekcheyev (1946), P. P. Lazarev (1947), S. V. Kravkov (1950), according to when eye sensitivity to light at the level of complete adaptation to the dark reflected not only processes in the receptor itself but also the state of the visual centers in the cerebral cortex and the brain stem. Accordingly, a change in the level (efficiency) of eye sensitivity to light as a result of an irritant can be regarded as reflecting physiological shifts of both peripheral and central origin.

Selecting three of the five subjects who were most sensitive with respect to odor perception and using an ADM adaptometer according to a generally accepted method, the author investigated the reflex effect of an acetone-phenol mixture on eye sensitivity to light under conditions of adaptation to the dark. All three subjects had normal vision and a comparatively stable dark-adaptation curve. In making the ADM test the fact was considered that the odor perception threshold of the selected most sensitive testers coincided with that previously ascertained (Yu. G. Fel'dman, 1962; B. M. Mukhitov, 1963) and as a point of departure adopted the same authors' results for the thresholds of acetone and phenol reflex action on eye sensitivity to light.

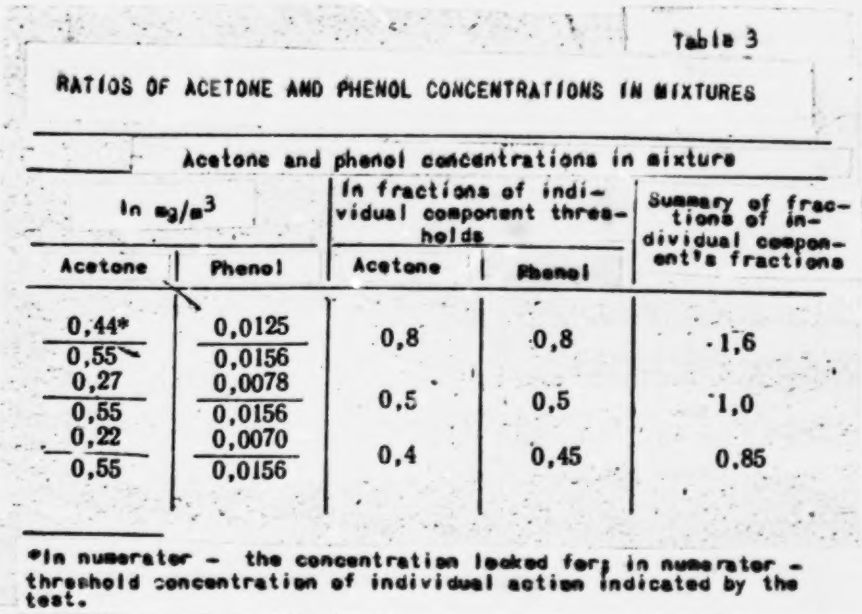

Table 3

RATIOS OF ACETONE AND PHENOL CONCENTRATIONS IN MIXTURES

Acetone and phenol concentrations in mixture				
In mg/m^3		In fractions of individual component thresholds		Summary of fractions of individual component's fractions
Acetone	Phenol	Acetone	Phenol	
$\frac{0,44^*}{0,55}$	$\frac{0,0125}{0,0156}$	0,8	0,8	1,6
$\frac{0,27}{0,55}$	$\frac{0,0078}{0,0156}$	0,5	0,5	1,0
$\frac{0,22}{0,55}$	$\frac{0,0070}{0,0156}$	0,4	0,45	0.85

*In numerater - the concentration looked for; in numerater - threshold concentration of individual action indicated by the test.

The first combination, with a total-concentration index of 1.6, produced statistically reliable changes in all three testers; when the index dropped to 1.0, only two persons responded, and at 0.85 none reacted. Thus, it appears that the reflex-action threshold of acetone and phenol in combination is a combined-concentration index of 1.0. Brief inhalation of the combined vapors produced contrasting effects in the testers, the eye sensitivity to light of R. L. and Ye. S. G. L. increasing. Electroencephalography (EEG) has been used broadly in experiments designed to determine norms for atmospheric pollutants. The EEG method adequately satisfies the requirements inherent in the theoretical and practical problems of pollution standards, being a highly sensitive indicator of the nervous system functional state. Reflexive changes in the electrical activity of the brain of three testers' inhaling the acetone-phenol mixture were also noted by making quantitative analysis of the natural alpha-rhythm amplification (A. D. Semenko, B. N. Balashov, Ye. V. Arzamastsev, 1963).

The threshold concentration of acetone and phenol affecting energy brain potentials have been set at 0.44 mg for the former (Yu. G. Fel'dman,

1962) and 0.15 mg/m^3 for the latter (B. M. Mukhitov, 1963). These were accepted as initial values in the present study, which were established from desynchronization of the alpha-rhythm in generating an electrocortical conditioned reflex. The present studies were based on the EEG dependence on the functional state of the brain. Primary attention was given to the alpha-rhythm peaks and to changes in the background of the peak as a result of inhaling the mixture, using as the stimulant a rhythmically blinking light with a frequency equal to the optimum for the test person's alpha-rhythm.

For four weeks all the test persons underwent training, exhibiting well-defined alpha-rhythms and a definite dynamic sterotype. Basic observations began after it was noted that inhalation of clean air was accompanied by only few fluctuations. This level was reached by conducting the observations under standard, nonvarying conditions. During the experiment the test persons were housed in a dimly lit soundproof chamber, in a comfortable semirecumbent position, with electrodes fastened to the cranial surface; they breathed from a cylinder through which 30 libers of clean air passed per minute. Two pickups on the back of the hands recorded motor responses to stimulation. The test persons were given prior instruction. The EEG's were recorded in the following order: initial background with clean air feed; after 4 min, a 6 min inhalation of the mixture, followeded by a 6-min period of breathing clean air (recovery period). Each test lasted 18 min, including 3 min of conditioning. The light was flashed for 18 sec every minute, its intensity changing every 5 sec. In the intervals between flashes the testers voluntarily moved in the chair. Then sound of different intensity was introduced for 10 sec. For 7 sec after the sound stopped test persons waited for the light to go on.

Through the experiment the test persons were active mentally and physically, which prevented the development of phase states. Functional loads can induce serious changes in tissues and organs, and the loads used in this study in the form of light and sound, their differential analysis, and responses evoked by such stimulators created a background which brought out shifts caused by reflex action of low acetone and phenol concentrations not perceived by taste, and which had no effect on eye sensitivity to light. These changes occurred against a background of amplified natural alpha-rhythm. Electric potentials were recorded by a bipolar method, on a 16-lead EEG manufactured by the Galileo company, from the frontal, temporal, and parietal regions of both sides of the brain. Responses to changes in the intensity of the light and sound stimulants, pressure on a piezo pickup, were also recorded. The EEG of each test person was analyzed as to the magnitude of amplified natural alpha-rhythm in microvolts, i. e., the integrated energy of brain potentials. For purposes of comparative analysis the total amplitudes were expressed in percent, with 100 percent being the average total amplitude of the first 3 min (taken as the background) against which the total amplitude of the remaining minutes was measured (A. D.

Semenenko, 1963). The results of these tests were processed statistically, and levels of reliability were determined for the shifts that occurred. Ratios of the studied and threshold concentrations are shown in Table 4.

Table 4

RATIOS OF ACETONE AND PHENOL CONCENTRATIONS IN DETERMINING THRESHOLDS OF THEIR REFLEX ACTION ON ELECTRICAL BRAIN ACTIVITY

Concentration of acetone phenol mixture				
In mg/m^3		In parts of individual component thresholds		In summarized concn. of threshold con cn . parts of individual components
Acetone	Phenol	Acetone	Phenol	
$\frac{0{,}22^{*}}{0{,}44}$	$\frac{0{,}0078}{0{,}0156}$	0,5	0,5	1,0
$\frac{0{,}17}{0{,}44}$	$\frac{0{,}0068}{0{,}0156}$	0,39	0,44	0,83

*In numerator - the looked for concentration; in denominator - threshold con centration of in dividu al component threshold as determined by the test.

The mixture which had a total concentration index equalling 1 caused statistically reliable changes in the enhanced natural alpha-rhythm of all test persons as illustrated by Fig. 1.

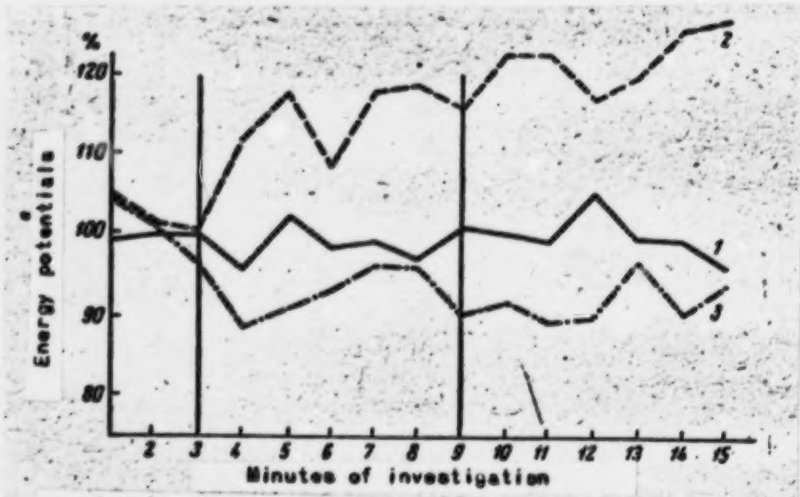

Fig. 1. Changes in brain enbrgy potentials in person R. L. effected by the inhalation of acetone-phenol vapor mixture.
1 - Clean air; 2 - 0.22 mg/m^3 acetone plus 0.0078 mg/m^3 of phenol; 0.17 mg/m^3 of acetone and 0.0068 of phenol

Concentrations with an index of less than 1 had no effect on the electrical activity of the brain.

Data of all 3 tests lead to the conclusion that the combined effect of acetone and phenol in inhaled air is equal to the summary effect of the individual admixture components.

If the threshold concentration determined by the EEG method is expressed as a fraction of the existing maximal allowable concentrations of the individual ingredients, the minimum effective total concentration will be 1.41; the maximum ineffective, 1.16. Accordingly, when acetone and phenol are simultaneously present in atmospheric air, the sum of their concentrations, expressed as a fraction of the allowable maximu, must not exceed 1.

To explain the resorptive action of low acetone and phenol concentrations in mixture, in determining their average daily permissible concentrations in air, 48 white male rats weighing 90-110 g were divided into 3 equal groups and subjected to 3-month chronic inhalation tests.

Rats of Group I were exposed to the inhalation of a mixture concentration 10 times higher than the maximum ineffective mixture; inhalation experiments were of short duration; the average was 1.657 ± .0215 mg/m^3 for acetone and 0.545 ± .0013 mg/m^3 for phenol. The total concentration index expressed as a fraction of the maximum allowable concentrations of the two chemicals individually was 10.19.

In the case of Group II rats, the acetone-phenol mixture contained: 0.1735 ± 0.00305 mg/m^3 of acetone and 0.00516 ± 0.0000769 mg/m^3 phenol and a concentration index of 1.012.

Rats of Group III served as controls.

During the inhalation and recovery periods the rats lived under identical conditions. Fresh air and air containing the acetone-phenol mixtures were run into the inhalation chambers at the rate of 35 li/min. Mixture content in the chambers was checked daily. Observation of the general condition and behavior of the rats during the inhalation experiment revealed no changes indicative of toxicity, and no symptoms indicating specific effects of acetone and phenol inhalation. Rats were weighed every 20 days. Rats of all 3 groups gained weight equally, but at the end of experiment and during the recovery period the controls lagged slightly.

During the chronic experiments the investigation included motor chronaxy of the antagonist muscles, blood cholinesterase activity, coproporphyrin concentration, and content of vitamins C, B_1, B_2 and N-methylnicotine amide--in the daily urine.

In choosing these resorptive action tests the author was guided by the assumption that this effect induced by small concentrations of injurious substances accumulated from general, nonspecific, sometimes monotypical and insignificant shifts which, as a rule, were functional and could be regarded as defense-adaptive responses (V. A. Ryazanov, 1964). Participation of central nervous mechanisms in the generation of these shifts must be considered general and obligatory.

One indication of a connection between the nerve centers and peripheral formations is control of subordination, i.e., regulation of the functional state of the periphery by the higher centers, a phenomenon which directly reflected in the level of many functional characteristics of the nervous and muscular apparatus, particularly its stimulability, one of the indexes of which is chronaxy: the rate of physiological response to stimulation. Here the primary difference appears in motor chronaxy of antagonistic muscles. The flexor centers are more easily stimulated than those of the extensors, i.e., the former have lower chronaxy than the latter. Given a normal relationship between the processes of stimulation and inhibition in the cerebral cortex, the proper ratio (as a rule greater than 1) of antagonist chronaxy will persist. This ratio changes under the effect of different environmental factors, the chronaxies converging, the ratio tending to become inverted. A telling symptom here is a weakening of the effect of the central nervous system, which is caused by development of the inhibitory process in the brain cortex.

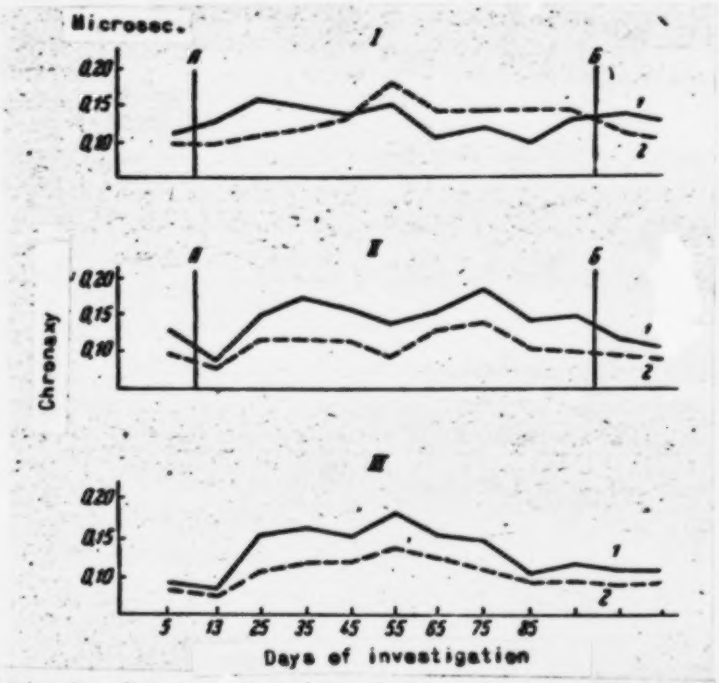

Fig. 2. Muscle antagonists chronaxy in rats affected by inhalation of air containing acetone and phenol mixtures (averages according to groups).
I, II, and III – Rat groups; 1 – Extensors, 2 – flexors
AB – Experimental inhalation period

Motor chronaxy was checked once every 10 days in 5 rats from each group. Curves in Fig. 2 show that rats of Group II and III exhibited normal ratios, while those in Group I on the 45th day of inhalation began to manifest a statistically reliable increase in correct flexor chronaxy as against that of the extensors. At the end of the recovery period the chronaxy ratio in rats of Group I returned to normal.

Establishing a biological balance between the demands of the organism and the enzyme system activity is one of the mechanisms of its adaptations to the conditions of existence. The enzyme systems participate in the biological and chemical transformations in the organism. One of these is cholinesterase, which hydrolized acetylcholine. Changes in the cholinesterase activity and cholinergic blood reactions reflected the functional state of nervous activity (D. Ye. Al'pern, 1958). Experiments by D. L. Pevsner (1954) showed that under conditions of normal relationships among basic nervous processes, such as stimulation and inhibition, the amount of blood cholinesterase activity did not fluctuate significantly. The predominance of the inhibitory process in the cerebral cortex corresponded to a marked drop in cholinesterase activity.

Blood cholinesterase activity was checked every 15 days by the method of J. Fleisher and E. Pope as modified by N. N. Pushkina and N. V. Klimkina (Pushkina, 1963). This parameter was checked in 5 rats from each group. On the 37th inhalation day a drop was noted in cholinesterase activity as indicated by R = 0.95. No such change was noted in rats of Group II (Fig. 3).

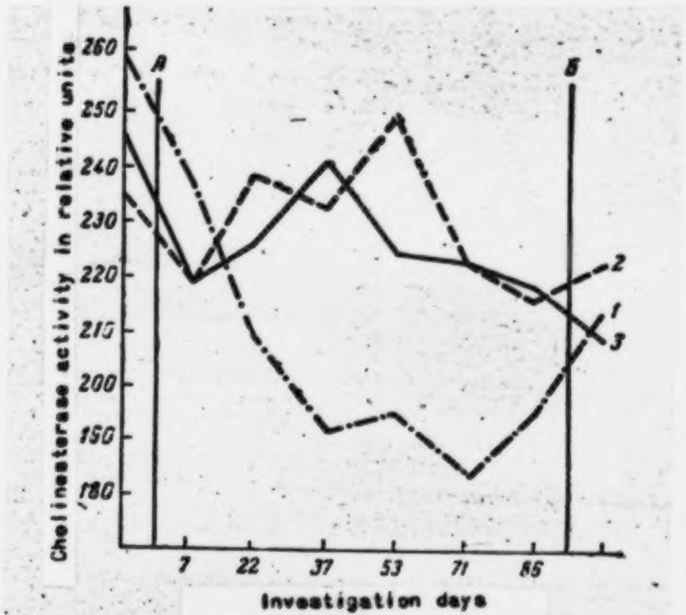

Fig. 3. Dynamics of blood cholinesterase activity in rats inhaling vapor mixture of acetone and phenol. (Group averages)
1 – Group I; 2 – Group II; AB – Inhalation periods.

No data were found in the literature dealing with the relative effects of low acetone and phenol concentrations on blood cholinesterase activity. According to B. A. Mukhitova (1963), small phenol doses lead to increased cholinesterase activity, while in the present experiment the opposite was true; this can be explained by the specific narcotic acetone action. M. Ya. Mikhel'son showed that narcotics reduced cholinesterase activity. However, since low concentrations had no specific effect, it would be more appropriate to ascribe these results to the nature of the combined acetone and phenol action and regard this as the result of an inhibitory focus arising in the cerebral cortex.

A substantial drop in blood cholinesterase activity was observed for brief periods in the course of the experiment. Obviously the compensatory mechanisms rapidly established a balance between the enzyme system and the requirements of the organism, caused by the effect of a chemical stimulant, the phenol-acetone mixture.

At the base of the energy conversion mechanisms in the living organism lie the processes of biological oxidation, i.e., of cellular respiration. Biological oxidation is an enzymic process, which is a complex of proteins and metallic porphyrins which create the condition for the blood respiratory function. Thus, porphyrins, as biologically active substances, are elements of such a vitally important pigment as hemoglobin. The human organism contains a series of porphyrin conversions, among them coproporphyrin with two of its isomers, I and III. Presence of porphyrins in the white matter of the brain and of the spinal cord is significant as a factor controling the character of the nervous system physiological processes. It is assumed that in these central nervous system regions where the cytochrome is either absent or occurred in insignificant quantities (the white matter of the brain) , porphyrins were responsible for the oxidizing processes. The supposition that porphyrins are good hydrogen acceptors and donors must be considered experimentally proved.

Clinical observations and experimental investigations of Yu. K. Smirnov (1953) emphatically suggest that porphyrin metabolism can be regarded as one indicator of the functional state of the central nervous system: enhanced porphyrin metabolism comes into play when the hypothalamic region of the brain is stimulated. Inhibitory processes in the brain reduced its functional activity and lead to a lowered porphyrin metabolism. All this supplants the assumption that determination of changes in the daily porphyrin content can be used as an objective test in determining the functional state of the central nervous system. For this reason the test has been used widely in experimental studies of the maximal allowable toxic substance concentrations in atmospheric air. With the aid of M. I. Gusev's and Yu. K. Smirnov's method (1960), the present author determined the total coproporphyrin content of the urine of 5 rats in each group. Porphyrin metabolism was below normal in rats of Group I; a statistically reliable change appeared in the 8th week of inhalation. At the end of the recovery period the coproporphyrin level was lower than that of rats in Groups II and III (Fig. 4). This decline must be explained as caused by disturbing the normal relationship between the basic nervous processes, as a physiological "means" of protecting the animals' organism.

Rates of vitamins C, B_1 , B_2, and N-methylnicotine amide elimination with the urine were studied next. Vitamins are catalyzers of biochemical living cell reactions. They participate largely in metabolism, of substances which constitute the enzyme systems. Accordingly determining vitamin content can offer supplementary evidence regarding the nature of the metabolic-enzyme processes. Literature reports describe vitamin metabolism disturbance among workers engaged in phenol and acetone production by the cumene method (N. N. Pushkina, 1964) at high concentrations of these substances in the shop air.

Vitamins were determined in 24-hour urine specimens daily in 5 rats from each group at 12 day intervals. Shifts in vitamin elimination by animals of the 3 groups were insignificant and statistically unreliable; on the 80th inhalation day the level of all the groups was nearly the same.

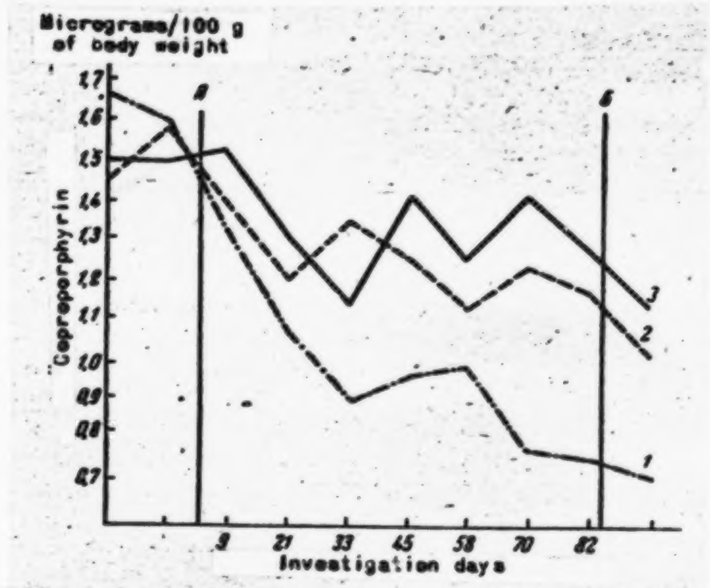

Fig. 4. Rate of coproporphyrin elimination via the urine in rats during inhalation of acetone-phenol vapor mixture inhalation

Notations same as in Figure 3.

Pathocytological tissue examination of rats of Group I showed vascular damage in some parenchymatous organs, edema of the stroma in the villi of the small intestines, and a change in capillary permeability with development of moderate perivascular and pericellular edema in the brain.

Thus, chronic exposure of Group I rats to the inhalation of air polluted with acetone and phenol simultaneously in concentrations (1.657 and 0.0545 mg/m^3) ten times in excess of the maximum inactive concentration of the mixture for short-duration exposure produced shifts which pointed to a reaction in the nervous system, viz., disturbance of the normal chronaxy ratio of the muscle antagonists, reduction in blood cholinesterase activity, a decrease in the rate of coproporphyrin elimination via the urine, and a series of minor pathomorphological changes. A phenol-acetone mixture in a concentration equal to the maximum active level (0.00516 and 0.1735 mg/m^3, respectively) produced no changes in rats of Group II.

Investigation of the acetone-phenol mixture resorptive effect also pointed to a complete summation of their individual effects. Therefore, it can be recommended that the maximum average-daily allowable concentrations for phenol and acetone in mixture be set at the single-exposure level, i. e., the sum of their fractions of the existing average-daily maximal allowable concentrations taken individually should not exceed 1.0.

In the spring of 1964 an investigation was made of the air around a synthetic phenol-acetone plant. Samples were taken on the leeward side 100, 300, 500, and 1000 m from the plant, 1-1.5 m above the ground. Results of simultaneously determined single-exposure concentrations of phenol and acetone are summarized in Tables 5 and 6.

Table 5

SINGLE ACETONE CONCENTRATIONS IN ATMOSPHERIC AIR AROUND PLANTS PRODUCING SYNTHETIC ACETONE AND PHENOL

Meters from discharge source	No. of air samples	No. of samples below method sensitivity	Mg/m^3	
			Maximal	Average
100	14	—	2,19	1,41
300	13	—	0,714	0,415
500	24	24	—	—

Table 6

SINGLE PHENOL CONCENTRATIONS IN ATMOSPHERIC AIR IN THE VICINITY OF PLANTS PRODUCING SYNTHETIC ACETONE AND PHENOL

Meters from discharge source	No. of air samples	No. of air samples below method sensitivity	Mg/m^3	
			Maximal	Average
100	17	—	0,323	0,187
300	13	—	0,2197	0,115
500	23	3	0,0869	0,039
1 000	25	25	—	—

At 100 and 300 m the acetone readings exceeded the maximum allowable (0.35 mg/m^3) by 6 and 2 times respectively; only at 500 m were no traces of the pollutants found. For phenol the maximum allowable 0.01 mg/m^3 concentration was exceeded at these three distances by multiples of 32, 22, and 8 correspondingly, and only at 1000 m were no traces of the pollutants found.

It is of some practical value to compare these data with the maximum allowable phenol and acetone concentrations when present as a mixture in doses in defined fractions of the maximal single-exposure concentration of each individually, the sum of which must not exceed 1. At 100 and 300 m the phenolacetone concentration exceeded the maximum allowable combined concentrations by factors of 38 and 24, respectively, indicating that the air surrounding this plant was heavily polluted, especially with phenol. Preventive measures should, therefore, be instituted first by freeing the gaseous emissions of phenol vapors.

The width of the sanitary protection felt around synthetic phenol-acetone plants can be determined reliably only by taking into account the effects of other gaseous pollutants discharged into the air by the plants.

CONCLUSIONS

1. Effect of an acetone and phenol mixture acting on the odor-perception organ, on eye sensitivity to light, and the electric activity of the brain, should be evaluated as the simple fractional sum of the individual components' effects.

2. Biological effects of the phenol and acetone mixture will appear when the fractional summation of their individual allowable maximal will exceed the value of 1.

3. The maximum permissible concentration of a phenol-acetone mixture in atmospheric air should be set at the biologically inactive concentration level for single exposure where the fractional summation of the acetone and phenol individual single allowable maximal levels did not exceed 1.

4. The average-daily allowable phenol and acetone combination can be set at the maximum single-exposure level.

DETERMINATION OF ALLOWABLE MAXIMUM CONCENTRATIONS OF PHENOL AND ACETOPHENONE WHEN SIMULTANEOUSLY PRESENT IN ATMOSPHERIC AIR

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The object of this study is to investigate the combined effect of phenol (Ph) and acetophenone (APh) vapors on the human organism. Such conbined effect occurs when the cumene method is employed in the production of phenol and acetone (AC), during which vapors of Ph, APh, acetone, benzene, and other toxic substances are emitted into the atmosphere. The effect of combinations of several of these poisons on the human organism may differ from that when the same substances are inhaled individually:

the result may be a total or partial summation, diminution or enhancement of the effect (N. S. Pravdin, 1929; N. V. Lazarev, 1938; V. A. Ryazanov, 1954). Actual final effect can be determined by experimental study of each. Most often the final effect is one of simple summation of the individual effects which can be expressed by the following formula

$$X = A/M_A + B/M_B$$

where A and B are the maximum allowable concentrations of the pollutant in mg/m^3 of air when they are present in the air individually.

The maximum allowable single-exposure and average-daily concentration for phenol and acetophenone in atmospheric air were set at 0.01 and 0.03 mg/m^3, respectively (B. M. Mukhitov, 1962; N. B. Imasheva, 1963). The toxicity of phenol has been adequately described elsewhere. It enters the organism in a variety of ways. Workers engaged in phenol production frequently manifest acute and chronic poisoning as a result of inhaling phenol vapors. The principal harm is first to the central nervous system, followed by the cardiovascular system; the activity of the gastrointestinal tract is damaged, and vitamin metabolism is impaired (V. K. Navrotskiy, 1928; Z. E. Grigor'yev, 1953; V. I. Petrov, 1960). A. S. Stegniy and Ye. K. D'yakonenko (1961) noted that workers exposed to phenol vapors developed hypotonia, polyneuritis, impairment of perception, absence of abdominal reflex, and inadequate convergence.

Little attention has been given to the toxic properties of phenol, and in the field which is related to this study only N. B. Imasheva's work fully explains the effects of low concentrations on the organism. Although phenol, which is widely used in industry, is not comparable to acetophenone which is given off as a byproduct. Under ordinary production conditions phenol and acetophenone do not affect the organism individually, but have a constant effect the nature of which has been investigated experimentally.

Two methods were used in determining phenol in atmospheric air: In a reaction with diazotized p-nitroaniline, which in a carbonic acid medium produced a yellow-green to red-brown color; this method has a sensitivity of 0.2 micrograms in 5 ml; and a more sensitive method involving 4-aminoantipyrine (V. A. Khrustaleva, 1962). Acetophenone was determined spectrophotometrically (M. D. Manita, 1963) by determining the optical density of an acetophenone solution in ethyl alcohol at a wavelength of 244 millimicrons in a cell with a layer thickness of 10 mm. The sensitivity of this method is 0.25 micrograms in 1 ml. The method of V. A. Khrustaleva (1961) was used in studying actual air pollution around a plant producing phenol and acetophenone. Effect of low phenol and acetophenone concentrations simultaneously present in the air was investigated by the methods of threshold of odor perception, eye sensitivity to light, and electrical activity of the cerebral cortex. Experiments were conducted with healthy persons. The threshold of phenol and acetophenone mixture odor perception were determined by a method recommended by the Committee on the Protection of Air Cleanliness. First, all subjects were familiarized with the odor of phenol and acetophenone mixture after which their threshold of odor perception to the individual mixture componenets was determined. Twenty-two persons cooperated, of whom two had a phenol odor perception of 0.017 mg/m^3 and a subthreshold of 0.016 mg/m^3; for 14 others the readings were 0.022 and 0.017 mg/m^3, respectively; while for the remaining 6 persons the threshold was high. B. Mukhitov set 0.022 mg/m^3 as the odor perception threshold for most sensitive persons. Ten persons had an average odor perception threshold of 0.010 mg/m^3 (subsensory, 0.008 mg/m^3); average readings for all others were 0.0285 and 0.01 mg/m^3, respectively; for one person the odor perception threshold lay above 0.03 mg/m^3. N. B. Imasheva set the odor perception threshold of acetophenone at 0.01 mg/m^3 for most sensitive persons.

Determination of odor perception threshold for phenol-acetophenone was conducted with the cooperation of 18 persons, 6 of whom were proved to be most sensitive. Altogether 752 tests were made. The summation concentration of the phenol and acetophenone mixture was expressed as a fraction of the threshold concentrations, as previously described. For most sensitive individuals the results of the two sets of tests were identical, i.e., a threshold of 0.022 mg/m^3 for phenol and 0.01 for acetophenone.

The odor perception threshold concentration of the mixture had a concentration summation of

$$0.99 \times \frac{0.013}{0.022}\ \text{mg/m}^3 + \frac{0.004}{0.01}\ \text{mg/m}^3,$$

i. e., when simultaneously present in atmospheric air the effect of low phenol and acetephenone concentrations is equal to the sum of their individual effects.

Expressed in terms of fractions of the individual thresholds, the undetectable mixture had summation concentration index of 0.72. The effect of low phenol and acetophenone concentrations on eye sensitivity to light was investigated using an ADM adaptometer and a method recommended by the Clean Air Committee. Some changes were made in cylinder design and air was run in at the rate of 30-35 li/min. The increased rate of air flow was not perceived by the subjects. Volume of air flow (500 ml/sec) was generally maintained at the level of normal breathing. Three test persons were chosen for adaptometric studies. Two mix-concentrations were used; four

45-min tests were made with each mixture. Ninety-three readings were taken. Eye sensitivity to light was checked every 5 min and the results processed statistically.

B. M. Makhrtiov and N. B. Imasheva obtained the following concentration summation index in their studies of phenol and acetophenone individually:

$$1.0\ \frac{0.00747}{0.0015}\ \text{mg/m}^3\ \text{(PH)} + \frac{0.00517}{0.01}\ \text{mg/m}^3\ \text{(AP) and } 0.77\ \frac{0.0059}{0.155}\ \frac{0.0039}{0.01}$$

The first mixture effected a statistically reliable change in all 3 test persons, the second had no effect on the behavior and the dark-adaptation curve (Fig. 1).

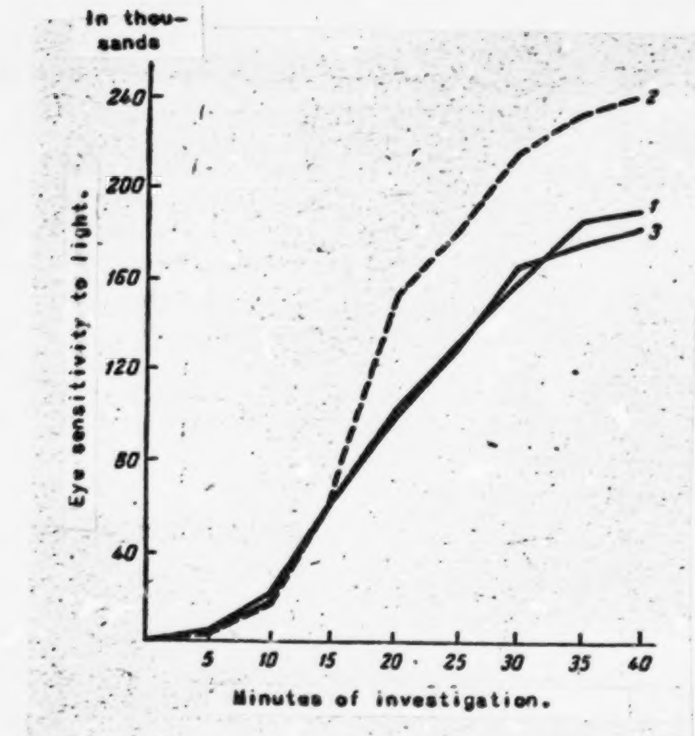

Fig. 1. Curve of eye adaptation to dark of female T. N. during the inhalation of acetone-phenol vapor mixture inhalation.
1 – Clean air; 2 – Mixture with a summation concentration index of 1; 3 – Summation concentration index of 0.77

As in the olfactory threshold tests complete simple summation of the effects prevailed here: the concentration summation index for the minimum effective mixture was 1.0.

The effect of low phenol and acetophenone concentration mixtures on the cerebrocortical activity using (1) a method developed by A. D. Semenko for hygienic investigations which involving amplification of the natural alpha-rhythm, while successively stimulating the subject with a flickering light at a frequency fitted to that of the subject's alpha-rhythm; and (2) a method similar to that described by U. G. Pogosyan. Altogether 112 tests were made on 3 test persons. Frontal, lobal, and parietal readings were taken from each hemisphere. Two gaseous phenol and acetophenone mixtures were used. The first was 0.00759/0.0156 mg/m^3 for phenol = 0.49 of the individual threshold concentration, and for acetophenone 0.0357/0.07 mg/m^3 = 0.51, and a fractional-concentration summation index of 1.0. In the second mixture the proportions were 0.00584/0.0156 (= 0.374) and 0.00276/0.007 (=0.394), and an index of 0.77 (Fig. 2). Results of EEG tests were also computed by the Mukhitov-Imasheva threshold method. The first mixture produced statistically reliable changes in all 3 test persons; the second, none, confirming the principle of simple fractional summation of the phenol and acetophenone individual limiting concentrations in atmospheric air.

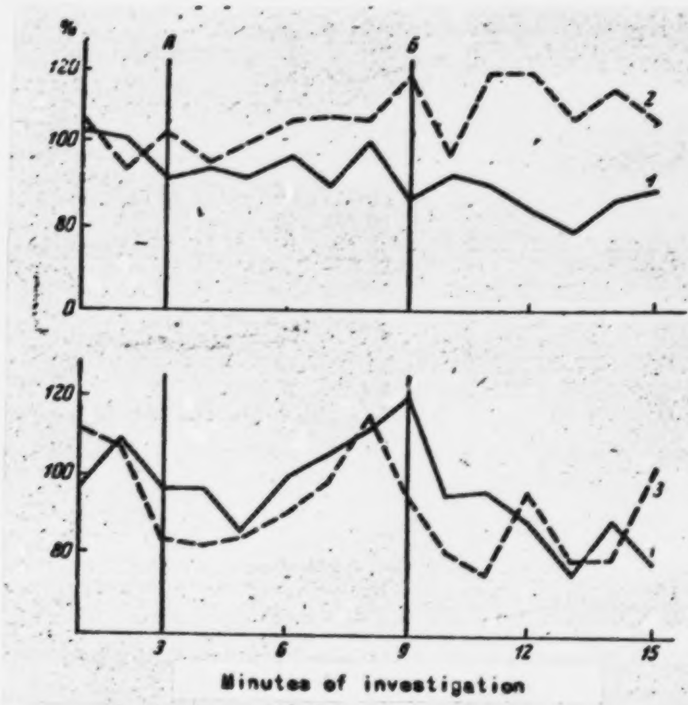

Fig. 2. Changes in electrical brain activity of Female T. N. effected by inhalation of air containing mixtures of phenol and acetophenone vapors
Notations same as in Fig. 1.
AB - period of vapor mixture inhalation

As a fraction of the individual maximum allowable single phenol and acetophenone concentrations the minimum effective mixture is 1.96, and the maximum ineffective is 1.51. Thus, when both substances are simultaneously present in atmospheric air, their fractional summation concentration must not exceed 1.5.

K. A. Bushtuyeva (1960), V. M. Styazhkin (1962) and B. K. Baykov (1964) investigated the combined effects of atmospheric pollutants; they also arrived at the fractional concentrations summation.

The resorptive action of phenol and acetophenone was studied next. Seventy-five white male rats weighing 70-100 g were housed as numerically equal groups in 5 inhalation chambers with the following pollutant concentrations. N. Z. Tkach cooperated with the author's partner in studying the effects of a triple mixture in Groups IV and V:

Group	Pollutant Concentration, in mg/m^3	Index of Individual Concentrations*
I	Acetophenone 0.0017, phenol 0.0062	1.19
II	Acetophenone 0.01732, phenol 0.0637	12.14
III	Controls	-
IV	Acetophenone 0.00147, phenol 0.0048, AC 0.136	1.36
V	Acetophenone 0.01141, phenol 0.04162, AC 1.334	11.76

*Decimal fraction of existing maximum allowable average-daily concentrations.

Concentrations were checked daily. During the inhalation period the general condition, weight, blood cholinesterase activity, motor antagonist chronaxy, porphyrin metabolism, eosinophile count in peripheral blood, and rate of 17-ketosteroids and vitamin C elimination with the urine were checked.

The four test groups differed in no way from the controls with respect to weight and general condition.

A slightly modified Fleisher-Pope-Spear method (1954) was generally used in determining cholinesterase activity. Rats of Groups II and V exhibited a statistically reliable reduction during the fourth week of inhalation which continued to the end of the exposure period; only during the recovery period did the cholinesterase-activity level return to normal. No negative effects were observed in the other groups (Fig. 3).

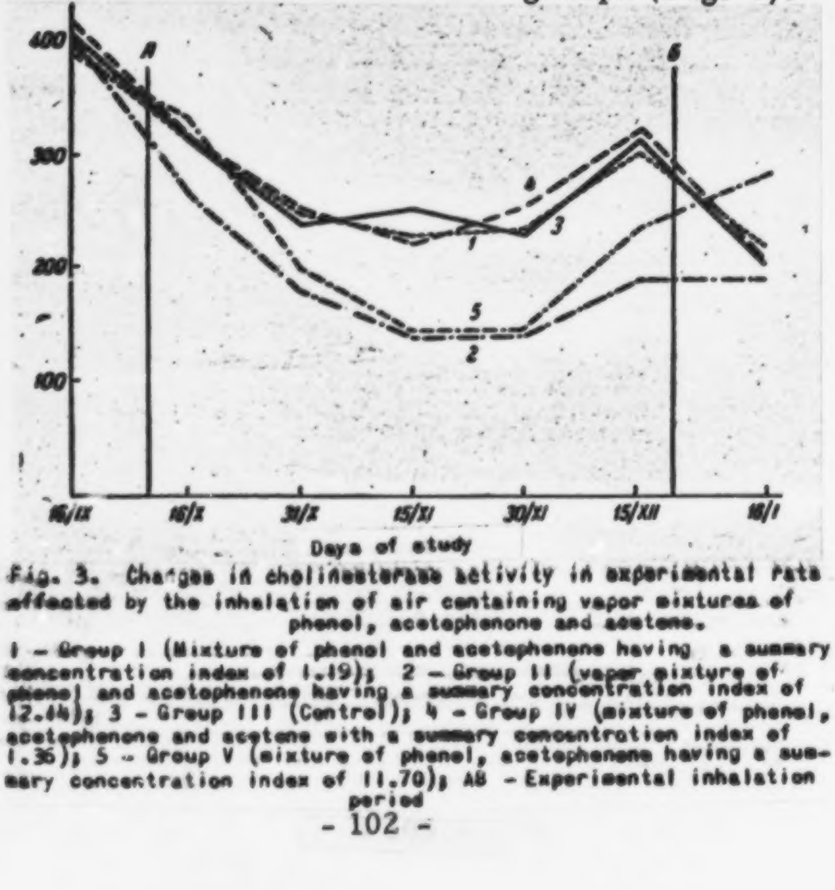

Fig. 3. Changes in cholinesterase activity in experimental rats affected by the inhalation of air containing vapor mixtures of phenol, acetophenone and acetone.

1 – Group I (Mixture of phenol and acetophenone having a summary concentration index of 1.19); 2 – Group II (vapor mixture of phenol and acetophenone having a summary concentration index of 12.44); 3 – Group III (Control); 4 – Group IV (mixture of phenol, acetophenone and acetone with a summary concentration index of 1.36); 5 – Group V (mixture of phenol, acetophenone having a summary concentration index of 11.70); AB – Experimental inhalation period

The nervous system condition was evaluated in the motor chronaxy of basic (antagonist muscles) shifts in which constituted an active index of stimulation states and which reflected changes in the external environment and the functional state of the organism with high precision. Exposure of the rats to high concentrations of the experimental gases (Groups II and V) had a significant effect on the functional state of the nervous system (Fig. 4), and induced changes at the end of the first month which disappeared at the end of the recovery period. The other groups manifested no changes in chronaxy ratio.

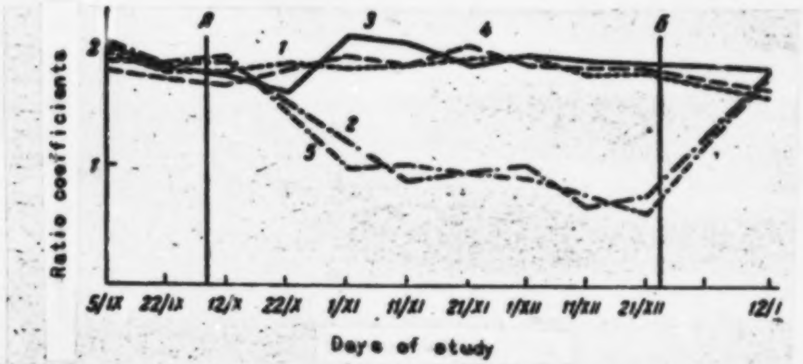

Fig. 4. Ratios of flexor and extnesor chronaxies in rats during periods of inhalation of air containing a mixture of phenol, acetophenone, and acetone vapors.
Notations same as in Fig. 3.

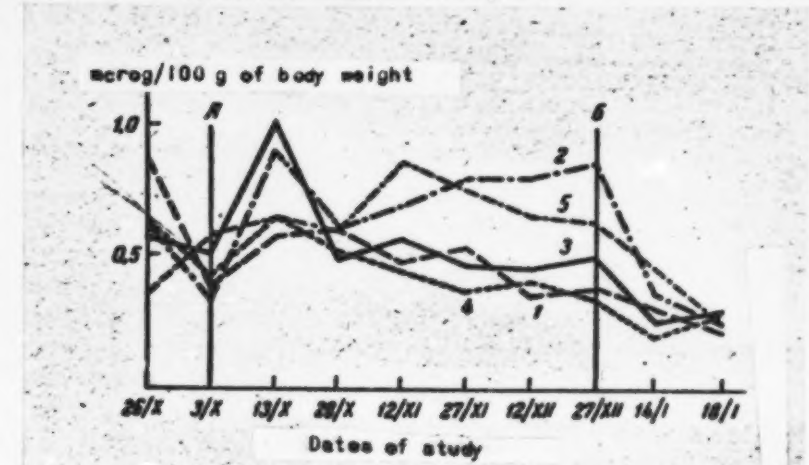

Fig. 5. Rate of coproporphyrin elimination *via* the urine during the inhalation of air containing a mixture of phenol, acetophenone and acetone vapors
Notations same as in Fig. 3.

Some intoxications and illnesses cause damage to the nervous system and lead to disturbed porphyrin metabolism. Elinination of coproporphyrine with the urine is an early indication of impaired nerve regulation in hemoglobin synthesis (O. M. Charnyy, S. E. Krasovitskaya, 1951). In the course of a 3-month experimental inhalation test, the experimental rats exhibited a statistically reliable increase in coprophyrin elimination *via* the urine.

During the restoration period this parameter returned to normal (Fig. 5).

Records were also kept of 17-ketosteroids elimination via the urine. The leading role in regulating excretion of steroid hormones of the adrenal cortex, which possess a well-defined reactivity, is played by the nervous system; the function of the adrenal cortex depends on a corticotropic hormone of the anterior hypophysis, which in turn is regulated by the central nervous system (S. G. Genes, 1955). The corticosteroid function responds to stimuli affecting the organism. Results of 17-ketosteroids investigations reflect damage done to the mechanisms which regulated the adrenal cortex function. This applies particularly to the eosinophiles. Eosinopenia indicates functional amplification in the adrenal cortex (D. Ya. Belov, 1957).

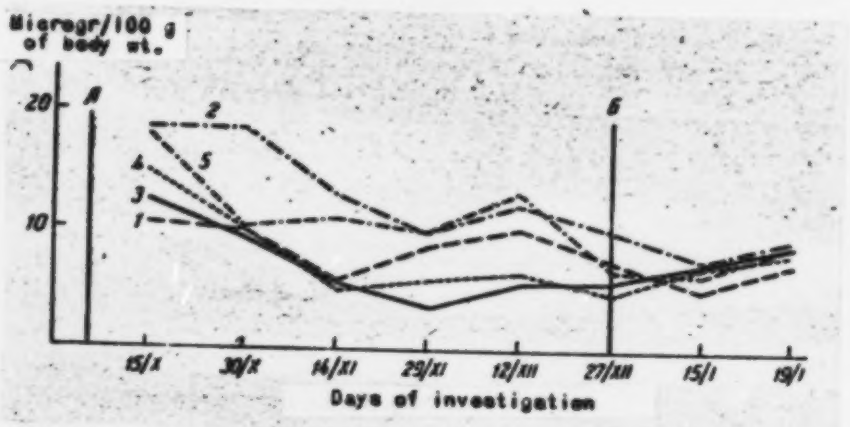

Fig. 6. Rate of ketosteroids elimination *via* the urine of rats exposed to the inhalation of air containing a mixture of phenol, acetophenone, and acetone vapors.
Notations same as in Figure 3.

In this connection a study was made of 17-ketosteroid secretion and hematic eosinophile count. Rats of Groups II and V exhibited a change in the first parameter throughout the inhalation period, which was statistically reliable only during the third month. This can be explained partly by the fluctuating 17-ketosteroid elimination in the control group (Fig. 6). Groups I and IV differed little from the controls. These tests were run every 15 days at the same hour of the day.

Daily intermittent analysis of eosinophile counts in the peripheral blood can present to the investigator a picture of the organisms organic reactivity. The most diverse effects leading to inhibition in the central nervous system provoke a drop in absolute eosinophile count (K. Kh. Kyrge, 1956). Counts were made in a Fuchs-Rosenthal chamber (S. M. Bakman, 1958). Rats of Groups II and V exhibited a reduced eosinophile count in the peripheral blood. Statistically significant eosinopenia appeared twice in the second and third month of experimental inhalation (Fig. 7). No statistically reliable changes were noted in the other groups.

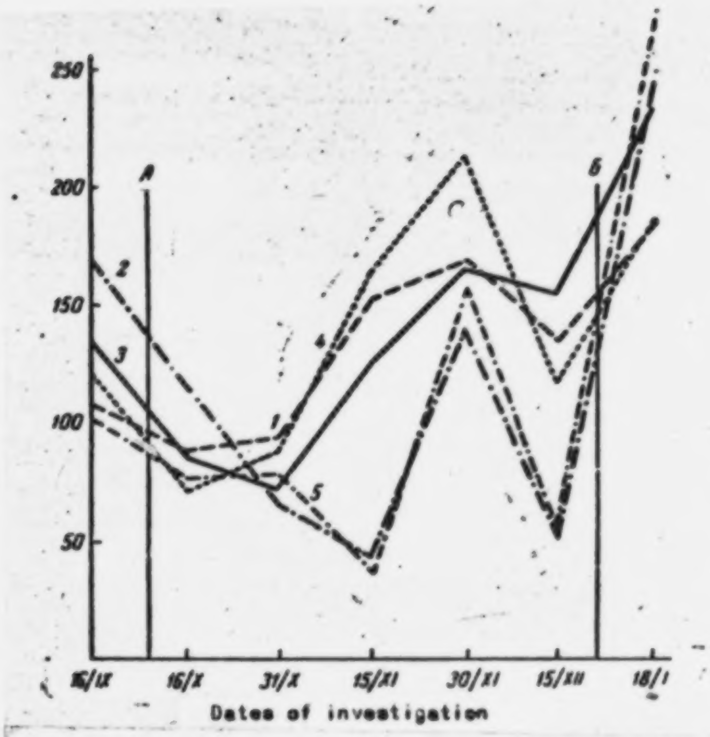

Fig. 7. Fluctuations in peripheral blood eosinophiles in rats subjected to the inhalation of air containing a mixture of phenol, acetophenone, and acetone vapors. Notations same as in Fig. 3.

Investigation of ascorbic acid content in the urine of the test animals revealed no deviations from the controls.

Thus, exposure of the rats to the inhalation of air containing phenol and acetophenone (Group II) and also acetone (Group V) affected cholinesterase activity, motor chronaxy of muscle antagonists, porphyrin metabolism, excretion of 17-ketosteroids with the urine, and eosinophile count in the peripheral blood. Fractional summation concentration of phenol and acetophenone at 1.19 index level in relation to their individual MAC and 1.36 when acetone was added provoked no organic changes even under conditions of chronic inhalation.

Taking into account the small difference between the maximum single-exposure to phenol-acetophenone concentration index here recommended (1.5) and the inactive average-daily concentrations investigation (1.19 and 1.36), as well as of the level of the minimum resorptively effective mixture (1.96), the present author accepts the recommendation of the Sanitary Air Section of the All-Union Study Commission that the average-daily concentration of the test gases be set the same as that for single exposure, viz., 1.5.

Part of the present study was devoted to an investigation of actual degree of air pollution in the vicinity of a synthetic phenol and acetophenone plant and hygienic assessment of the resulting data. Samples were collected in May 1964 on the leeward side of the plant at 100 to 1000 m. According to data of the municipal sanitation-and-epidemilogy station the plant discharged 2.2 metric tons of pure phenol into the air annually. Acetophenone discharges were not computed. At 500 m the maximum single phenol concentration exceeded the allowable maximum by a factor of 8.7; those of acetophenone by a factor of 9.3 (Table 3.) The combined concentration was 12 time above the recommended level. No traces of either gas were detected at 1000 m. The width of the sanitary break belt around a plant of similar type cannot be established without taking into account the entire complex of the toxic emissions.

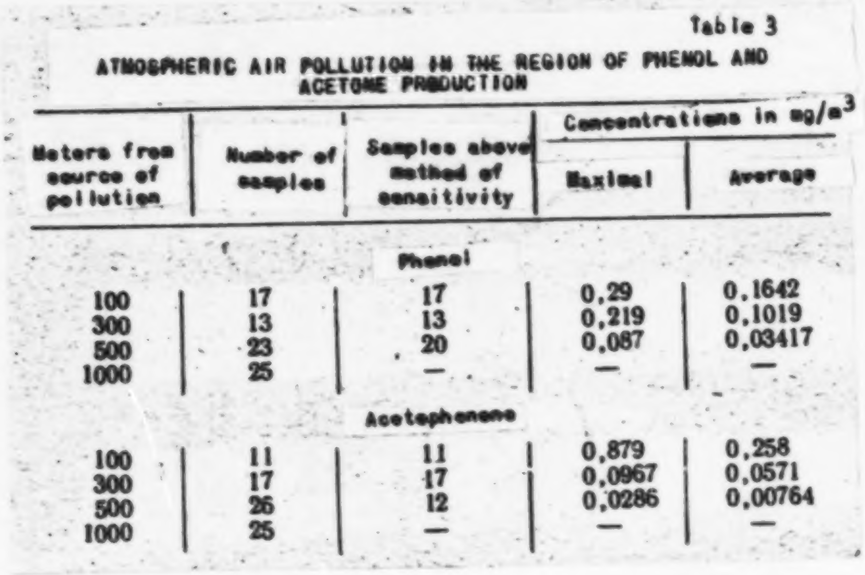

Table 3

ATMOSPHERIC AIR POLLUTION IN THE REGION OF PHENOL AND ACETONE PRODUCTION

Meters from source of pollution	Number of samples	Samples above method of sensitivity	Concentrations in mg/m^3	
			Maximal	Average
		Phenol		
100	17	17	0,29	0,1642
300	13	13	0,219	0,1019
500	23	20	0,087	0,03417
1000	25	—	—	—
		Acetophenone		
100	11	11	0,879	0,258
300	17	17	0,0967	0,0571
500	26	12	0,0286	0,00764
1000	25	—	—	—

CONCLUSIONS

1. The threshold odor perception concentration of the gas mixtures tested by most sensitive test persons was 0.013 mg/m^3 for phenol + 0.004 mg/m^3 for acetophenone with a fractional summation index of 1.0. An index of 0.72 (0.01 mg/m^3 phenol + 0.0026 mg/m^3 acetophenone was not perceptible.

2-3. The threshold concentrations for eye sensitivity to light in combination were as follows:

	Phenol	Acetophenone	Fractional Concentrations Summation Index	
			Min. Effective	Max Ineffective
Eye sensitivity to light	0.00747	0.00517 mg/m^3	1.0	0.77
Electrical Brain Activity	0.00759	0.00357 mg/m^3	1.0	0.77

4. The effect of phenol and acetophenone simultaneously present in the atmospheric air equals the simple sum of their individual effects.

5. Since the maximum allowable concentrations of phenol and acetophenone were established with some leeway, their maximum allowable combined single-exposure and average-daily concentrations, expressed as a fractional summation of the individual limit concentrations, such sum should not exceed 1.5. This also applies to a phenol-acetophenone-acetone mixture.

6. Inoculation of experimental animals with phenol-acetophenone and with phenol-acetophenone-acetone which have fractional concentration summation indices of 12.14 and 11.765, respectively, elicited significant changes in cholinesterase activity, motor antagonist chronaxy, porphyrin metabolish, 17-ketosteroid content, and caused pronounced eosinopenia. When the respective indices were lowered to 1.19 and 1.36, no such sequelle were noted.

7. The conbination of phenol and acetophenone, and their combination with acetone elicited no changes in the organism even upon prolonged (chronic) exposure to the inhalation of air containing simultaneously vapors of phenol and acetophenone or the two plus acetone when the fractional summation of their individual maximal allowable concentrations have indexes not exceeding 1.19 and 1.36 correspondingly.

8. This evaluation principle is applicable to investigations dealing with air containing three simultaneous pollutants.

9. The width of the sanitary protection zone in the case of plants producing synthetic phenol and acetone should be recommended by taking into account the total complex of pollutants emitted into the atmospheric air, and not on the basis of discharged phenol, acetophenone and acetone alone.

COMBINED EFFECTS OF LOW ACETONE AND ACETOPHENONE CONCENTRATIONS IN THE AIR OF THE LIVING ORGANISM

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The rapid development of the chemical industry has been accompanied by increased discharges into the atmosphere of a great volume of toxic complexes of broad contaminant spectra. A most frequently encountered combination consists of acetone (AC) and acetophenone (AP), by products of the cumene method for the production of synthetic phenol (PH) and acetone (AC) simultaneously. Acetophenone in this case is a product of secondary reactions; it is also continuously discharged into the atmosphere. Results of previous studies indicated that the combined effect of these pollutants were equal to the fractional summation of the effects of the individual components: sulfur dioxide + sulfuric acid aerosol (K. A. Bushtuyeva, 1960); chlorine + hydrogen chloride (V. M. Styazhkin, 1962); and carbon disulfide + hydrogen sulfide (B. K. Baykov, 1963).

The purpose of the present study was to determine the effects of low acetone and acetophenone concentrations on the living organism. Both poisons attack principally the central nervous system. Considerable study has been devoted to the toxicology of acetone (A. Ye. Yefremov, 1929; N. F. Okuneva, 1930; I. D. Mishenin, 1933; I. S. Tsitovich, 1935; P. M. Sukhov, 1935; N. V. Lazarev, 1954; Yu. G. Fel'dman, 1960; Ch. Khadnan' et al., 1962; and others), whereas the literature on acetophenone is inadequate (Laborde, 1885; S. S. Kamenskiy, 1889; Friedman and Maase, 1910; N. B. Imasheva, 1963; et al.) and deals with the effects of high toxic concentrations, except the work of Fel'dman on acetone and Imasheva on acetophenone which deal with low concentrations under conditions of brief and chronic exposure. No studies were found in the literature which dealt with the combined effects of acetone and acetophenone.

M. I. Ol'shanskaya and V. V. Likhacheva (1935) investigated the combined effects of acetophenone and benzene vapors on doves in concentrations of 25,000 and 10,000 mg/m^3. The authors investigated the fractional summation of the individual effects and found the most powerful mixture to be 1:1. T. A. Shtessel' (1937) conducted 2- and 4-hr experiments with white mice by administering to them combinations of acetone and methyl alcohol, acetone and ethyl ether, acetone and toluene, and acetone and benzene and found that the principle of simple summation prevailed in all cases.

The critical concentration of individual or combined substance were regarded as the one which caused the experimental mice to assume a lying-down lateral position.

Nischiama and Isami (1957) noted intensification of the toxic effects of acetone and benzene, and a leucocyte count drop to a greater degree than when the two are administered individually. The experiment began by determining the threshold concentration of acetone and acetophenone odor perception individually and in combination. Acetone was determined nephelometrically, the sensitivity of which was 1 microgram per 4.5 ml. Acetophenone was determined by a spectrometric method of 0.25 micrograms per ml sensitivity.

The odor perception threshold concentration for acetone was determined in 22 healthy persons aged 18-48 years, using the commonly accepted method (V. A. Ryazanov, K. A. Bushtuyeva, Yu. V. Novikov, 1957). All told, 412 observations were made at concentrations of 2.0, 1.654, 1, 481, 1.096, and 0.80 mg/m^3. Results summarized in Table 1 show that for the most sensitive test persons the threshold was 1.096, while 0.80 was the highest subthreshold concentration.

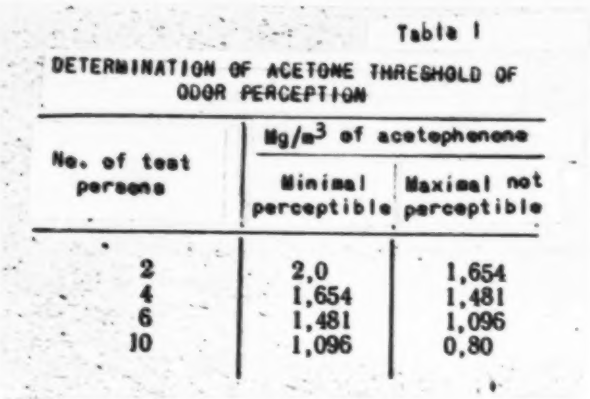

Table 1

DETERMINATION OF ACETONE THRESHOLD OF ODOR PERCEPTION

No. of test persons	Mg/m^3 of acetophenone	
	Minimal perceptible	Maximal not perceptible
2	2,0	1,654
4	1,654	1,481
6	1,481	1,096
10	1,096	0,80

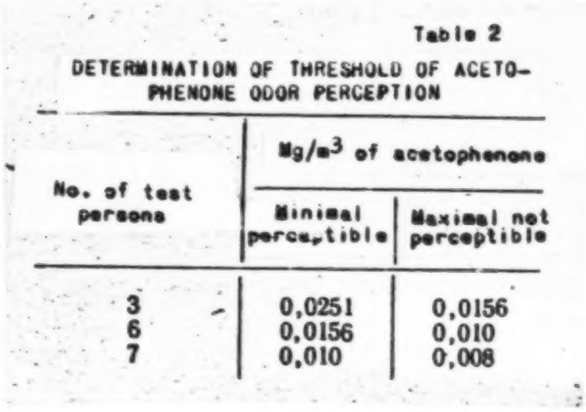

Table 2

DETERMINATION OF THRESHOLD OF ACETOPHENONE ODOR PERCEPTION

No. of test persons	Mg/m^3 of acetophenone	
	Minimal perceptible	Maximal not perceptible
3	0,0251	0,0156
6	0,0156	0,010
7	0,010	0,008

In the present study four acetophenone concentrations were used in 216 tests and 16 test persons. Results presented in Table 2 agree with those of Fel'dman, who set the acetone threshold concentration at 1.1 mg/m^3, and Imasheva, who set the acetophenone threshold concentration at 0.01 mg/m^3.

Odor perception thresholds for mixtures of different concentrations were investigated by conducting 265 tests on 16 persons. A fractional concentration summation index equaling 1.0 indicated simple summations between 1 and 2 indicated partial summation; less than 1 reflected an increase in the order of magnitude. Table 3 shows that the majority of the test persons recognized the second mixture by its odor (fractional concentration summation index = 1.03-1.26) Only the most sensitive test persons detected the third mixture (index = 1.01).

Table 3

DETERMINATION OF THRESHOLD ODOR PERCEPTION OF ACETONE AND ACETOPHENONE VAPOR COMBINED

	Mixture			
	I	II	III	IV
Acetone (mg/m^3)	1,096	0,711	0,565	0,348—0,711
Acetophenone (mg/m^3)	0,010	0,0061	0,005	0,004—0,0061
Fractional summation of individual threshold	От 1,06 до 2,0	От 1,03 до 1,26	1,01	От 0,67 до 0,88
Number of test persons perceiving the odor	16	12	5	—
Number of test persons not perceiving the odor	—	4	11	16

Data in Table 3 also show that the minimum perceptible mixture is an acetone and acetophenone concnetration of 0.565 + 0.005 mg/m^3 concentrations respectively, with a summation index of 1.01. The mixture cannot be detected if the index is less than 1. In other words, the effect of acetone and acetophenone is equal to the sum of their individual effects, a phenomenon applicable to less sensitive test persons as well.

The functional state of the central nervous system was determined by the eye sensitivity to light method as indicated by eye adaptation to dark. This test is used widely in arriving at standards for atmospheric pollutants, using physiological reflex reactions as indexes of effects of toxic substance concentrations not perceptible by odor but affect the physiological state of the cortex. Results of animal behavior tests with subthreshold pollutant concentrations were reported by V.A. Gofmekler (1960), Yu. G. Fel'dman (1960), R. Ubaydullayev (1961), and others.

In her studies of combined effects of sulfur dioxide and sulfuric aerosol acid, K. A. Bushtueva discovered the phenomenon of physiological summation: when these compounds were administered simultaneously, eye sensitivity to light enhanced to a degree, amounting to a simple summation of the effects produced by the two contaminants individually.

B. K. Baykov (1963) described the fractional summation phenomenon in a study of the effects of combined carbon disulfide and hydrogen sulfide. The 3 test persons who cooperated in this study were most perceptive to odors ranging between 24 and 27 years of age. The standard method was used with which 42 tests were made using two mixtures of AC + AP, mg/m^3: 0.283 + 0.005, and 0.223 + 0.0037, corresponding to summation concentration indices of 1.01 and 0.77, respectively. According to Fel'dman and Imasheva, threshold concentrations of reflex action of acetone and acetophenone on eye sensitivity to light for the most sensitive testers were 0.55 and 0.01 mg/m^3. Only the first mixture index = 1.01 was statistically signicant, and the reactions of the subjects were quite different. In one the sensitivity rose; in another it dropped; in the latter it rose at first during the 20th minute, then dropped sharply during the 25th minute; by the end of the experiment, it returned to normal. Table 4 presents eye adaptation to light data for the three test persons during the 20th minute.

Table 4

ADAPTOMETRIC TEST RESULTS

Test person's initials	Mg/m³	Eye sensitivity to light on the 20th adaptation minute
	Acetone-acetophenone	
S. P.	Clean air	81 475
	0,283 + 0,005	103 050 (b)*
	0,223 + 0,0037	82 133 (o)
S. L.	Clean air	83 225
	0,283 + 0,005	111 000 (b)
	0,223 + 0,0037	82 133 (o)
Ya. T.	Clean air	95 550
	0,283 + 0,005	74 175 (c)
	0,223 + 0,0037	90 033 (o)

*Statistical reliability; b - 99%; c - not reliable

The mixture of low acetone and acetophenone concentrations having a fractional concentration summation under 0.77 proved physiologically inactive and elicited no statistically significant shifts or changes, indicating that the principle of fractional concentration summation also prevailed in adaptometric studies. The investigation included a study of the effect of subthreshold acetone and acetophenone concentrations jointly on the electrical cerebrocortical activity by the alpha-rhythm method (A. D. Semenko, 1964). A similar method has been described by U. G. Pogosyan and Yu. S. Korneyev. Tests were conducted with cooperation of three persons using a "Galillo" polyphysiograph, making 62 readings. Records were made of the temporal and frontal biocurrents of both hemispheres and their combination, and of the central parietal part of the left hemisphere. A statistical plot was made of the temporal, most sensitive data. Here, as before, two acetone + acetophenone mixtures were tested (mg/m^3): 0.22 + 0.0035, and 0.18 + 0.0027 in which the Fel'dman and Imasheva thresholds were 0.44 and 0.007 mg/m^3, respectively, and which correspond to fractional concentrations summation indices of 1.0 and 0.78. The effect of each mixture was tested 4 or more times with regular intermittent clean air breathing intervals.

The first mixture increased the cerebral potential energy in two test persons and decreased it in the third. Statistically significant changes appeared on the 3rd and 4th inhalation minutes. Data obtained with the second mixture did not differ significantly from those with clean air, showing that the principle of simple fractional concentrations summation also operated in the case of subthreshold stimulation. Accordingly, the fraction summation index for the first acetone-acetophenone mixture of threshold concentrations in terms of mg/m^3 should be computed as follows:

$$\frac{0.22}{0.35} + \frac{0.0035}{0.003} = 0.63 + 1.16 = 1.79 \quad \text{and} \quad \frac{0.18}{0.35} + \frac{0.0027}{0.003} = 0.51 + 0.9 = 1.41$$

for the present mixture.

On the basis of the above data, it can be suggested that the maximum concentration summation index of AC and AP in atmospheric air should not exceed 1.5.

The resorptive effects were studied using 45 white male rats which were divided into 3 equal groups and exposed to chronic inhalation for 84 days. The initial weight of the rats ranged between 80-100 g. The average concentrations of acetone and acetophenone in mg/m^3 were as follows:

Group I--1.855±0.168; Group II--0.192±00197; Group III--Controls.

Indices of maximum allowable concentrations for rats inhaling mixture 1 was 10.9 and rats inhaling mixture 2 was 1.9. Adjusting the Group II index on the basis of the thresholds of individual components and results of most sensitive EEG test persons reduced the index to 0.71. Accordingly, animals of Group II inhaled a subthreshold mixture and Group I inhaled a mixture 10 times as concentrated.

Indices of effects in this study were general condition and weight; motor antagonist chronaxy; cholinesterase activity; rate of coproporphyrin elimination; neutral 17-ketosteroids, and Vitamin C elimination with the urine; and changes in absolute eosinophile count in the peripheral blood.

In the course of the inhalation experiment rats in all groups were active and gained weight uniformly. Yu. M. Uflyand (1941), A. N. Magnitskiy (1948) and others found that there was a direct relationship between the functional state of the central nervous system and the motor chronaxy level. Through subordinating relationships the former regulated the activity of the peripheral nerve apparatus and of the deeper lying nerve centers. The subordinating brain effect is reflected in the constancy of normal extensors and flexors chronaxy ratios of 1.5:1, 2:1, and 2.5:1. Motor chronaxy was checked in 5 rats of each group 3 times each month using an ISE-01 electron pulse stimulator. Statistically reliable ratio changes appeared in rats of Group I beginning with the 4th week, chiefly as a delay in the flexor chronaxy. An inverse ratio appeared at the beginning of the 6th week, after which the curve flattened out and returned to the physiological norm only during the recovery period. In rats of Group II no such distortions were noted. Group I changes can be interpreted as reflecting inhibition in the central nervous system, involving a complex system of cortical and subcortical subordination centers. The presence of these processes is confirmed by depression of whole blood cholinesterase activity of the test animals.

According to modern concepts, stimulus transmission from a nerve to a muscle involves the acetylcholin system, i.e., cholinesterase.

Here acetylcholin plays the part of mediator: an excessive accumulation of it in the organism leads to reduced lability of nerve process and to development of inhibition (I. N. Volkova, 1954; D. Ye. Al'pern, 1963).

Reductions in enzyme activity as a result of prolonged exposure to various chemical compounds have been noted by several authors (N. B. Imasheva, 1963; V. A. Chizhikov, 1964; A. V. Mnatsskanyan, 1964; et al.). In this study cholinesterase activity was determined by the J. Fleischer and E. P. Pope method (1954) based on changes in the intensity of solution coloration as a result of changes in the amount of acetic acid formed during acetylcholin chloride hydrolysis. Color intensity was determined photoelectrocolorimetrically. Twice monthly, 5 rats of each group had their cholinesterase activity checked by the usual tail vein method.

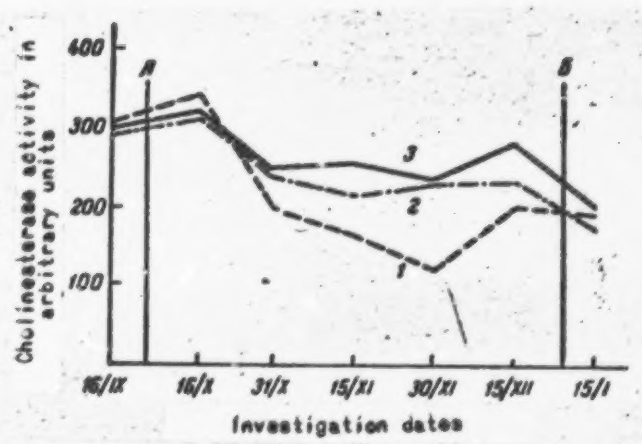

Fig. 1. Blood cholinesterase activity changes in rats of different groups
1 - Group I - Acetone + acetophenone of fractional summation index 10.9; 2 - Group II - acetone + acetophenone of fractional summation index 1.2; 3 - Group III - Controls; AB - Period of inhalation

Curves in Fig. 1 show a significant reduction in enzyme activity which appeared by the end of the first month of inhalation in rats of Group I and continued to the end of the second month. By the end of the inhalation period a trend toward normalization had set in, but the level was still depressed as compared with the controls. Statistical reliability index of these changes was 95-99 percent. No statistically reliable changes were noted in rats of Group II throughout experimental inhalation periods.

Porphyrin metabolism was evaluated on the basis of its excretion rate with the urine. A. M. Charnyy and Se. E. Krasovitskaya (1951) noted a direct relationship between porphyrin metabolism and serious disturbances in the central nervous system. The first author suggested that the porphyrins may participate in the redox processes of those parts of the brain in which cytochrome and cytochrome oxydase were missing. Watson (1941) established a relationship between rate of porphyrin excretion from the organism and erythropoetic activity in the bone marrow, and M. I. Gusen (1960) was first to introduce porphyrin metabolism in his hygienic studies. For 6 months the present author administered lead oxide to rabbits in a 10 microg/m^3 concentration and discovered that the amount of coproporphyrin in the urine doubled as compared with the control animals. Disturbed porphyrin metabolism has also been noted by other authors engaged in chronic administration of low concentrations of atmospheric pollutants (G. I. Solomin, 1962; D. G. Odoshashvili, 1962; V. A. Chizhikov, 1964; et al.).

The present author used an SF-4 spectrophotometer in the UV region (M. L. Gusev, and Yu. I. Smirnov, 1960). Twenty-four hour urine specimens had been collected every 15 days from 5 mice in each group, making 33 readings -- 9 before, 18 during, and 6 after inhalation. Analysis of the results showed that the combined effect of acetone and acetophenone in concentrations of 1.855 and 0.0168 mg/m^3, respectively, caused a definite shift in the porphyrin metabolism of Group I rats.

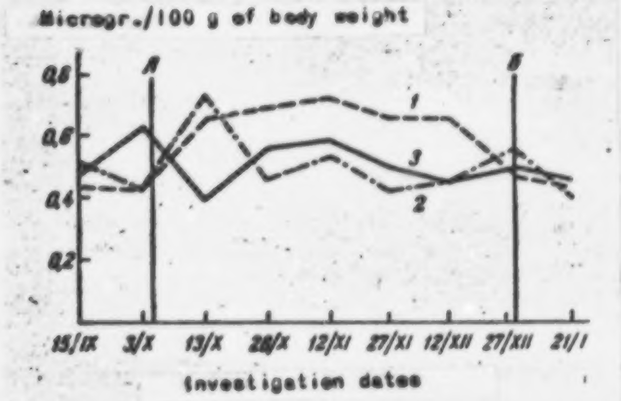

Fig. 2. Rate of coproporphyrin elimination *via* the urine of rats inhaling acetone + acetophenone vapor.
Notations same as in Fig. 1.

Curves in Fig. 2 show that beginning with the 4th week of experimental inhalation, coproporphyrin metabolism increased noticeably and rose to a statistically significant level during the second inhalation period (R = 0.95). Rats of Group II exhibited no substantial deviations from the controls.

The hormonal system activity encompassing the anterior lobe of the hypophysis and the adrenal cortex is regulated by the central nervous system through the hypothalmus. High concentrations of some chemical compounds elicited nonspecific response in this system, namely reduction in the absolute eosinophile count in the blood and an increase in neutral 17-ketosteroids in the urine (Ye. I. Spynu, 1962; L. E. Gorn, 1963). In this connection the present author attempted to find nonspecific reactions as a result of low acetone and acetophenone concentrations. 17-ketosteroids were determined by the O. M. Uvarovskaya method (1956) with check tests made twice monthly for a total of 24 tests. Curves in Fig. 3 show that the 17-ketosteroid level dropped in rats of Group I during the early days of the inhalation and sharply rose beginning with the 40th day. During this period the 17-ketosteroid level in the control group was 6.85 microg per 100 g of body weight, while in rats of Group I it was 17.41, a rise that continued until the 70th day, after which it returned to the control levels. These changes were statistically reliable.

The fluctuations in 17-ketosteroid content in rats of Group II did not differ substantially from those of the control group.

Eosinophile counts in the blood were made by the S. M. Bakman (1958) method. Twice a month, at the same hour of the day, blood was taken from 5 rats in each group; a total of 99 readings were made. After 10 days of inhalation the eosinophile count in rats of Group I dropped somewhat, apparently as a result of the initial reaction of the organism to acetone and acetophenone (Fig. 4). It dropped sharply later to a lower fluctuating level which persisted to the end of the inhalation experiment. The reliability index of the drop was 0.95. The osinophile count in rats of Group II approximated that of the controls, although on the 55th day there was an increase that was not statistically reliable. A. Ye. Kulakov (1965) also observed eosinopenia in animals chronically receiving low concentrations of hexamethylenediamine.

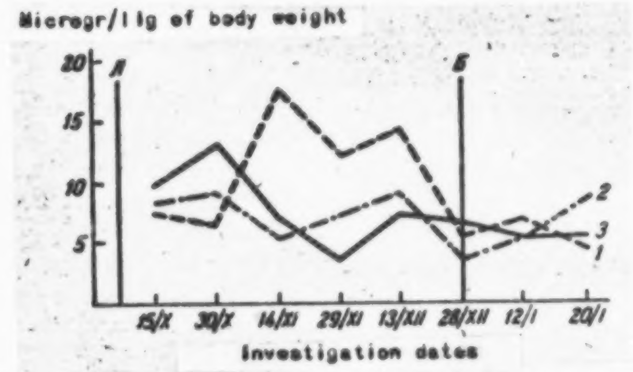

Fig. 3. Content of neutral 17-ketostyroids in the urine of rats of different Groups
Notations same as Fig. 1.

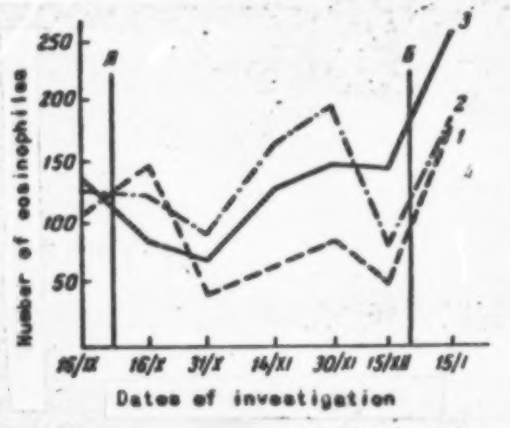

Fig. 4. Actual number of blood eosinophiles of rats exposed to the inhalation of air containing acetone and acetophenone in low concentrations
Notations same as in Fig. 1.

Thus, the investigations point to the high sensitivity of the tests employed and bring out the nonspecific organism reactions to unfavorable effects of atmospheric pollutants. Records of vitamin C elimination rate through the experimental inhalation showed no statistical significant changes; in other words, the vitamin C balance remained undisturbed. Based on results of the foregoing tests it is recommended that 1.2 be adopted as the maximum allowable fractional concentrations summation index for acetone and acetophenone combination in atmospheric air. However, in view of the wide range of unknown values between the threshold (10.9) and subthreshold (1.2) concentrations the Committee Section on The Protection of Air Cleanliness resolved to set the average-daily allowable index at the same level as the single-exposure, ivz., 1.5.

In addition to above experiments with humans and animals a survey was made of single acetone and acetophenone concentrations in the air around a plant producing synthetic phenol and acetone, taking 131 samples at 100 to 1000 m from the plant.

Data in Table 5 show that at 100-300 m from the plant there was considerable acetone air pollution: in 27 samples acetone concentrations substantially exceeded the maximal allowable 0.35 mg/m^3. Only at 500 m were all 24 samples negative for acetone.

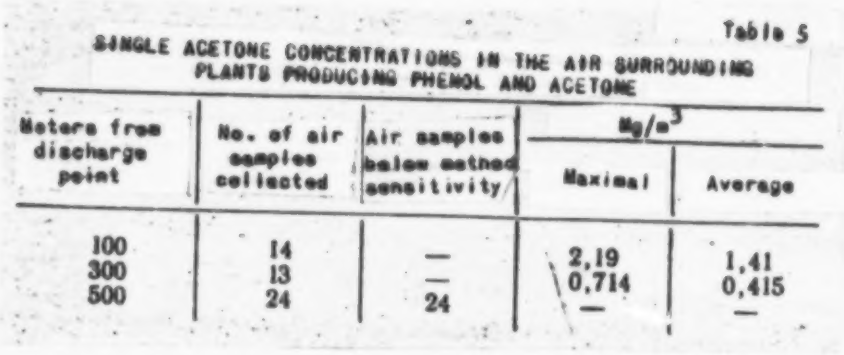

Table 5

SINGLE ACETONE CONCENTRATIONS IN THE AIR SURROUNDING PLANTS PRODUCING PHENOL AND ACETONE

Meters from discharge point	No. of air samples collected	Air samples below method sensitivity	Mg/m^3	
			Maximal	Average
100	14	—	2,19	1,41
300	13	—	0,714	0,415
500	24	24	—	—

Data in Table 6 show that acetophenone was present in significant concentrations in samples collected at 100-500 m from the plant, the maximum concentrations exceeding the allowable maximum of 0.003 mg/m^3. At 500 m 46 percent of the samples exceed the allowable maximum. Only at 1000 m were all the air samples negative for acetophenone.

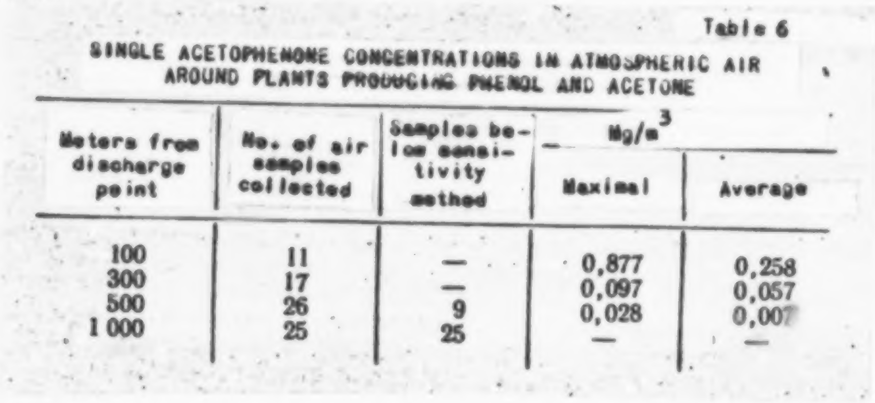

Table 6

SINGLE ACETOPHENONE CONCENTRATIONS IN ATMOSPHERIC AIR AROUND PLANTS PRODUCING PHENOL AND ACETONE

Meters from discharge point	No. of air samples collected	Samples below sensitivity method	Mg/m^3	
			Maximal	Average
100	11	—	0,877	0,258
300	17	—	0,097	0,057
500	26	9	0,028	0,007
1 000	25	25	—	—

Accordingly, the sanitary protection zone for this type of manufacture must be not less than 1000 m wide.

CONCLUSIONS

1. On the basis of odor perception threshold, the minimum perceptable acetone and acetophenone concentrations individually should be for the most sensitive persons, 1.096 and 0.01 mg/m^3, respectively.

2. Where acetone and acetophenone are simultaneously present in an air sample the intensity of their combined odors is subject to simple summation.

3. The reflex effect of acetone and acetophenone in simultaneous combination on eye sensitivity to light and on electrical brain activity can be expressed in terms of complete summation.

4. Chronic 24-hour daily exposure of white rats to the inhalation of air containing a mixture of acetone and acetophenone the fractional concentrations summation index of which is equal to 10.9 will bring about functional shifts in the organism of the experimental rats expressed as a lowering in the muscle antagonist chronaxy control by the central nervous activity, in the central control of cholinesterase activity of the blood, will enhance the rate of coproporphyrin elimination via the urine, and will give rise to many nonspecific reactions originating in the kidney cortex, probably due to increased 17-ketosteroid content in the urine and reduced number of blood eosinophiles. No physiological changes were noted in experimental rats which inhaled air containing a mixture of acetone and acetophenone the summation index of which was 1.2.

5. The maximal single and average 24-hour fractional concentrations summation index of acetone and acetophenone simultaneously present in atmospheric air must not exceed 1.5.

STUDIES IN THE STANDARDIZATION OF MAXIMUM ALLOWABLE HYDROGEN FLUORIDE CONCENTRATIONS IN THE AIR OF INHABITED AREAS

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Many publications appeared in the Soviet and foreign literature which deal with toxicology of fluorine (F) and the clinical course of industrial fluorosis (Ye. Ya. Girskaya, 1959; et al.). While the effects of F on drinking water have been given adequate treatment, the same cannot be said for small concentrations of F dumped into the air as a result of industrial emissions.

For the past several years this author investigated the levels of atmospheric pollution near aluminum and cryolite plants in the Urals, particularly the zonal distribution of F in the air at different heights (M. S. Sadilova, 1958, 1959, 1964), accompanied by a survey of the health of children who live in these areas. In F-industry districts the inhalation exhibited a general tendency toward illness and toward diseases of the respiratory organs; the noted F-specific diseases were mottling the enamel of the teeth, and increased F secretion via the urine. Children living near aluminum-producing plants had abnormally high F content in the bones. These observations showed that F in atmospheric air must be considered as an important factor in the pathology of the juvenile population (M. S. Sadilova, 1957, 1962; A. F. Aksyuk and G. V. Bulychev, 1962). The continued growth in the number of aluminum, cryolite, superphosphate, and other similar plants made it imperative that the maximal allowable concentration of F in the air be established.

S. V. Miller (1955) recommended such a level on the basis of calculations, starting from the maximum allowable concentration of F in drinking water (1.5 mg/l), on the assumption that F compounds in water and air were toxic in the same degree. Taking 1.5-2 liters as the amount of drinking water consumed per day and 15-20 cu m as the amount of air passing through the lungs every 24 hours, Miller calculated that the F concentration in air ought not to exceed 0.15 mg/m^3. Considering the fact that F had an unfavorable effect on living conditions in areas where industrial plants discharged F, Miller recommended a maximal single concentration of 0.03 mg/m^3 and an average 24-hour daily concentration of 0.01 mg/m^3. This recommendation was adopted.

The present author investigated the effects of hydrogen fluoride (HF), which is the most toxic compound contained in industrial discharges. The effects of HF and F-containing dust (individually and in combination) will be discussed later. The odor perception threshold for HF was determined experimentally by a method commonly employed in the Soviet Union. Concentration constancy was checked by sampling the air before and after a test.

A total of 672 tests was made with 17 persons of normal odor perception. Test concentrations of 0.02 to 0.22 mg/m^3 were used, and results showed that the minimum perceptible concentration ranged between 0.03 and 0.11; for the majority of the subjects (10) the threshold was 0.03. The subthreshold concentration was 0.02 mg/m^3 (Table 1).

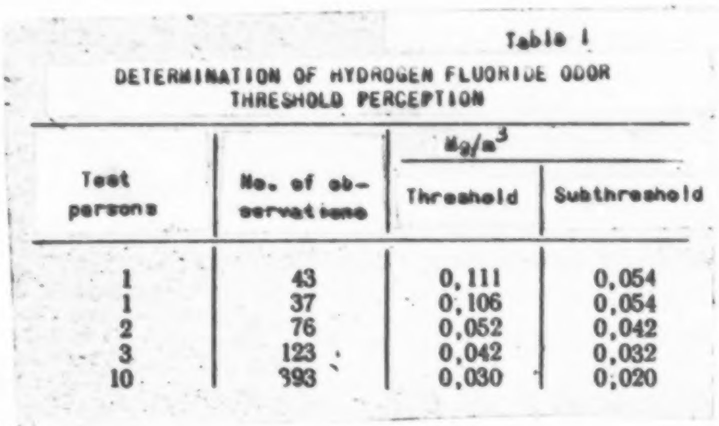

Table 1

DETERMINATION OF HYDROGEN FLUORIDE ODOR THRESHOLD PERCEPTION

Test persons	No. of observations	Mg/m^3	
		Threshold	Subthreshold
1	43	0,111	0,054
1	37	0,106	0,054
2	76	0,052	0,042
3	123	0,042	0,032
10	393	0,030	0,020

A determination was made next of HF effects on the central nervous system by way of the upper respiratory tracts. Changes in eye sensitivity to light were recorded in 3 test persons with normal vision. Resulting concentrations were 0.02, 0.03, and 0.06 mg/m^3. The latter two concentrations, and especially the last, produced a marked increase in light sensitivity, while clean air and the lowest concentration proved inactive. These results were statistically reliable. The light=sensitivity data for one of the three test persons are presented in Fig. 1.

Thus, the odor perception threshold and the threshold of reflex action of HF on the functional state of the cerebral cortex, as recorded by the adaptometer method, were on the same level, viz., 0.03 mg/m^3. The results clearly point to the fact that HF belonged to substances having a trigeminal effect. Animals were exposed to experimental inhalation of HF unterruptedly for 5 months on specially constructed chambers. Hydrofluoric acid vapor in appropriate concentrations in air was run into the inhalation chambers at the rate of 18 li/min. The following HF concentrations were used in the tests: 0.10, 0.03, and 0.01 mg/m^3. By using a 40 percent hydrofluoric acid as the stock solution, the above HF-air mixtures could be obtained by diluting the 40% stock solution according to the following ratios: 1:27, 1:500, and 1:40,000, respectively. Air samples from the chambers were collected in a cylinder with a No. 2 ashless membrane filter and subsequently passed through two consecutive absorbers containing twice-distilled water. F content was determined by S. K. Chirkov's alizarin-thorium method (1957), which has a sensitivity of 0.001 mg/m^3. HF-concentrations were checked 5 times a day in chambers I and II, and 4 times a day in chamber III. No significant fluctuations were observed.

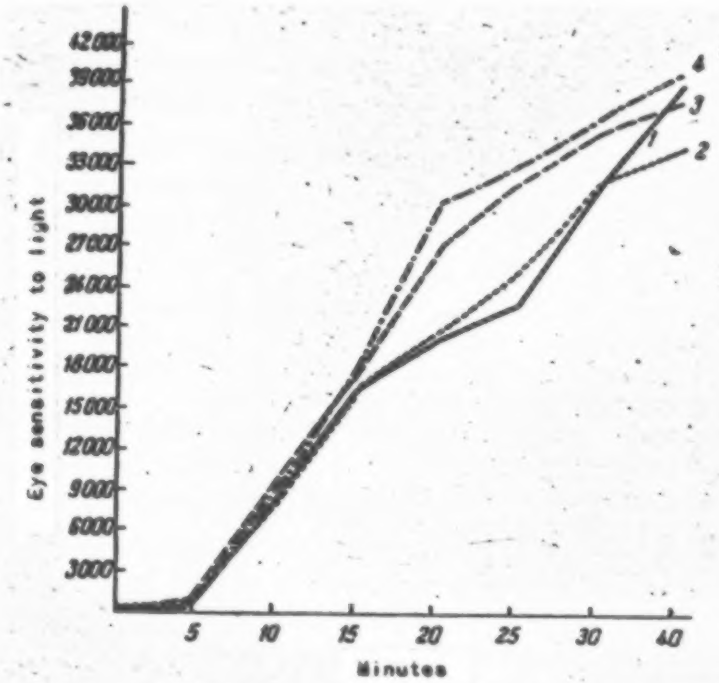

Fig. 1. Eye sensitivity to light in one test person who inhaled clean air (1), air containing 0.02 mg/m^3 of hydrogen fluoride (2), 0.03 mg/m^3 (3) and 0.06 mg/m^3 of hydrogen fluoride (4)

Each chamber contained 19-22 white male rats 2 months old. HF fluctuations were as follows (in mg/m^3: Group I--0.10±0.002; Group II--0.03±0.0001; Group III--0.01±0.00009; Group IV--control.

The F content in the rats' diet was standard: wheat bread--0.13 mg %; black bread--0.36 mg %; cabbage--0.17 mg %; oat grain--0.17 mg %; dry matter, mild--0.14 mg/li. The general condition, weight, and state of peripheral blood were observed before and during the experiment. It was noted that during the first inhalation month a large share of the Group II rats became agitated, attacked each other, and behaved aggressively toward the experimenter. Afterwards they returned to normal behavior. No changes in weight were observed. Hemoglobin, erythrocytes, reticulocytes, leucocytes, leucocyte formula of the peripheral blood did not substantially deviate from the normal. Special attention was given to the functional state of the central nervous system of the experimental animals, measuring it from the conditioned reflexes and chronaxy. We also utilized neurohistological methods. These tests were run on 8-11 animals in each group.

During the chronic experiments, special attention was paid to the functional state of the central nervous system which was studied by the method of condition reflexes and motor chronaxy supplemented by neurohistological studies. Rats in the Kotlyarev chamber were trained to two positive conditioned reflexes, a bell and a red light, and one negative, a buzzer. After the positive reflexes were established, a sterotype developed, consisting of 7 signals in a given order: bell, light, light, bell, buzzer, bell, light. The effect of F on conditioned reflex activity was judged by the rate of reflex formation and reinforcement, the number of correct responses, and the latency period duration. In developing the sterotype the state of differentiating inhibition, successive inhibition, and the character of the strength correlations of the reflexes to stimuli of varying strength were taken into consideration. The chronic effects of HF in the two lower concentrations were also studied against a background of added functional loading.

The results showed that serious disturbances in conditioned reflex activity developed as a result of HF inhalation in 0.10 mg/m^3 concentration such as delayed reinforcement of positive reflexes, inadequate reflex stability, and prolonged latent period (Table 2).

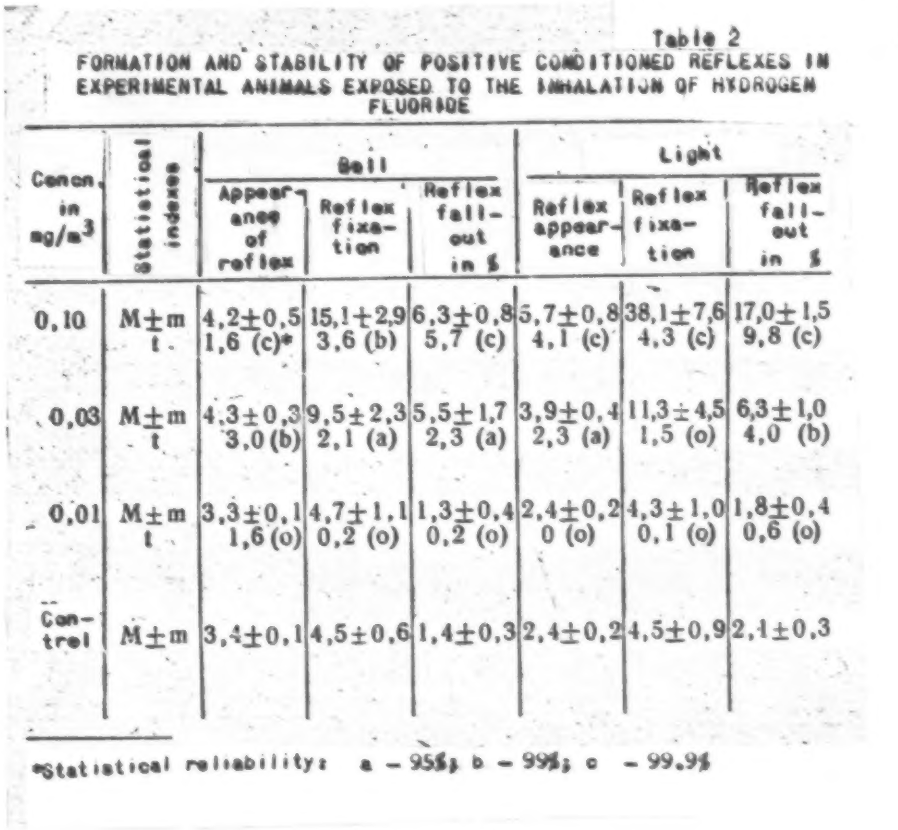

Table 2

FORMATION AND STABILITY OF POSITIVE CONDITIONED REFLEXES IN EXPERIMENTAL ANIMALS EXPOSED TO THE INHALATION OF HYDROGEN FLUORIDE

Concn. in mg/m^3	Statistical indexes	Bell			Light		
		Appearance of reflex	Reflex fixation	Reflex fall-out in %	Reflex appearance	Reflex fixation	Reflex fall-out in %
0,10	M±m t	4,2±0,5 1,6 (c)*	15,1±2,9 3,6 (b)	6,3±0,8 5,7 (c)	5,7±0,8 4,1 (c)	38,1±7,6 4,3 (c)	17,0±1,5 9,8 (c)
0,03	M±m t	4,3±0,3 3,0 (b)	9,5±2,3 2,1 (a)	5,5±1,7 2,3 (a)	3,9±0,4 2,3 (a)	11,3±4,5 1,5 (o)	6,3±1,0 4,0 (b)
0,01	M±m t	3,3±0,1 1,6 (o)	4,7±1,1 0,2 (o)	1,3±0,4 0,2 (o)	2,4±0,2 0 (o)	4,3±1,0 0,1 (o)	1,8±0,4 0,6 (o)
Control	M±m	3,4±0,1	4,5±0,6	1,4±0,3	2,4±0,2	4,5±0,9	2,1±0,3

*Statistical reliability: a – 95%; b – 99%; c – 99.9%

In developing the stereotype in the experimental rats there appeared instances of successive inhibition and disinhibition of the differentiation in comparison with the groups of rats subjected to lower concentrations and with the controls. The force of the conditioned reflexes stimulation and response reaction lost its impact as shown by the appearance of compensating and paradoxical phases.

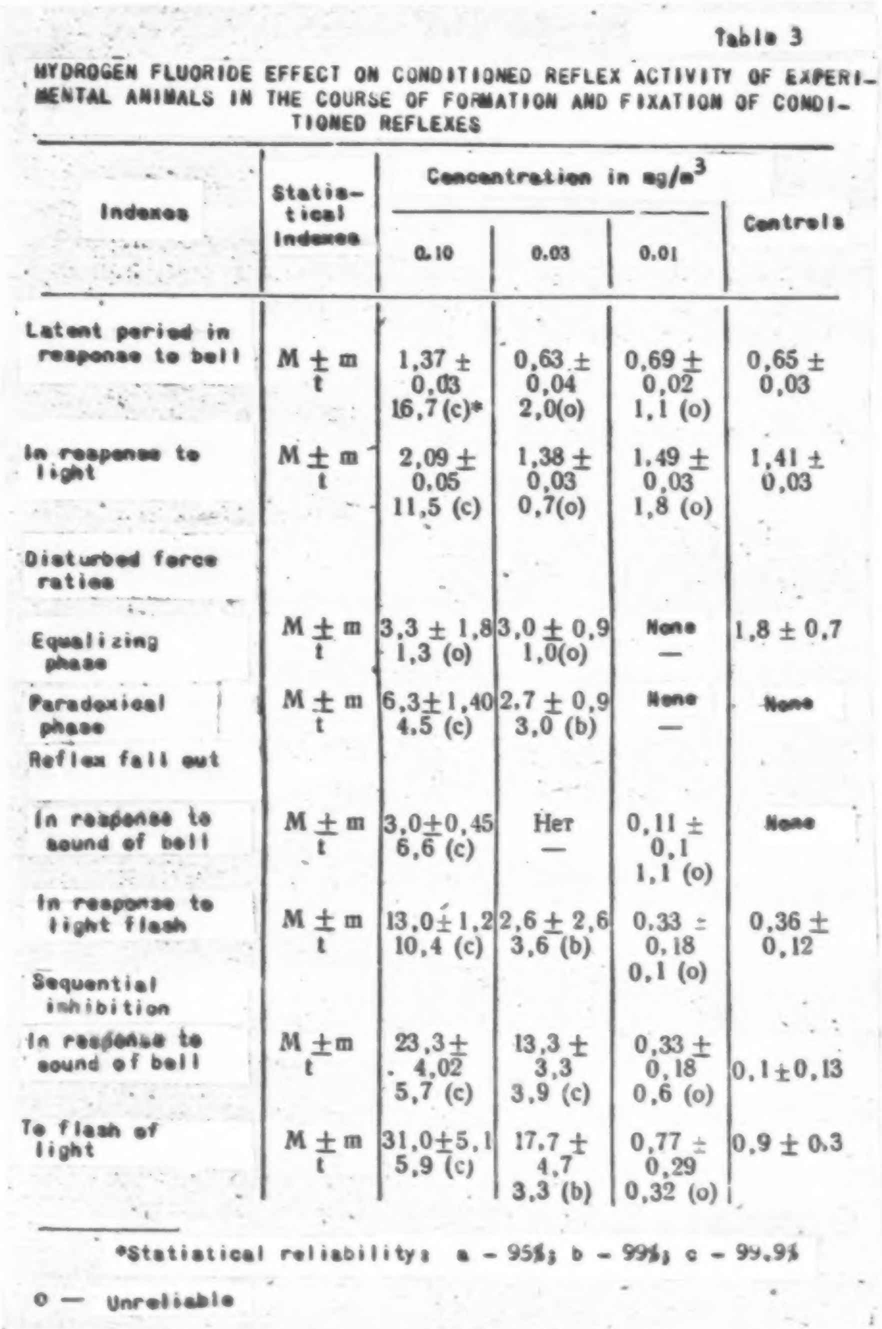

Table 3

HYDROGEN FLUORIDE EFFECT ON CONDITIONED REFLEX ACTIVITY OF EXPERIMENTAL ANIMALS IN THE COURSE OF FORMATION AND FIXATION OF CONDITIONED REFLEXES

Indexes	Statistical indexes	Concentration in mg/m^3			Controls
		0.10	0.03	0.01	
Latent period in response to bell	M ± m t	1,37 ± 0,03 16,7 (c)*	0,63 ± 0,04 2,0(o)	0,69 ± 0,02 1,1 (o)	0,65 ± 0,03
In response to light	M ± m t	2,09 ± 0,05 11,5 (c)	1,38 ± 0,03 0,7(o)	1,49 ± 0,03 1,8 (o)	1,41 ± 0,03
Disturbed force ratios					
Equalizing phase	M ± m t	3,3 ± 1,8 1,3 (o)	3,0 ± 0,9 1,0(o)	None —	1,8 ± 0,7
Paradoxical phase	M ± m t	6,3± 1,40 4,5 (c)	2,7 ± 0,9 3,0 (b)	None —	None
Reflex fall out					
In response to sound of bell	M ± m t	3,0±0,45 6,6 (c)	Нет —	0,11 ± 0,1 1,1 (o)	None
In response to light flash	M ± m t	13,0± 1,2 10,4 (c)	2,6 ± 2,6 3,6 (b)	0.33 ± 0,18 0,1 (o)	0,36 ± 0,12
Sequential inhibition					
In response to sound of bell	M ± m t	23,3 ± 4,02 5,7 (c)	13,3 ± 3,3 3,9 (c)	0,33 ± 0,18 0,6 (o)	0,1 ± 0,13
To flash of light	M ± m t	31,0±5,1 5,9 (c)	17,7 ± 4,7 3,3 (b)	0,77 ± 0,29 0,32 (o)	0,9 ± 0,3

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*Statistical reliability: a - 95%; b - 99%; c - 99.9%

o — Unreliable

Changes in conditioned reflex activity were also noted in rats which inhaled 0.03 mg/m^3 concentration of HF, especially in the presence of functional load under such conditions there appeared an increase in the number of conditioned reflex fall outs and in the number of successive inhibition cases. The 0.01 mg/m^3 HF concentration elicited no changes in conditioned reflex activity.

At the end of the month's recovery period the conditioned reflex activity of Group I rats somewhat improved but remained different from the controls, while that of Group II returned to normal. Results of muscle antagonist chronaxy studies are presented graphically in Fig. 2.

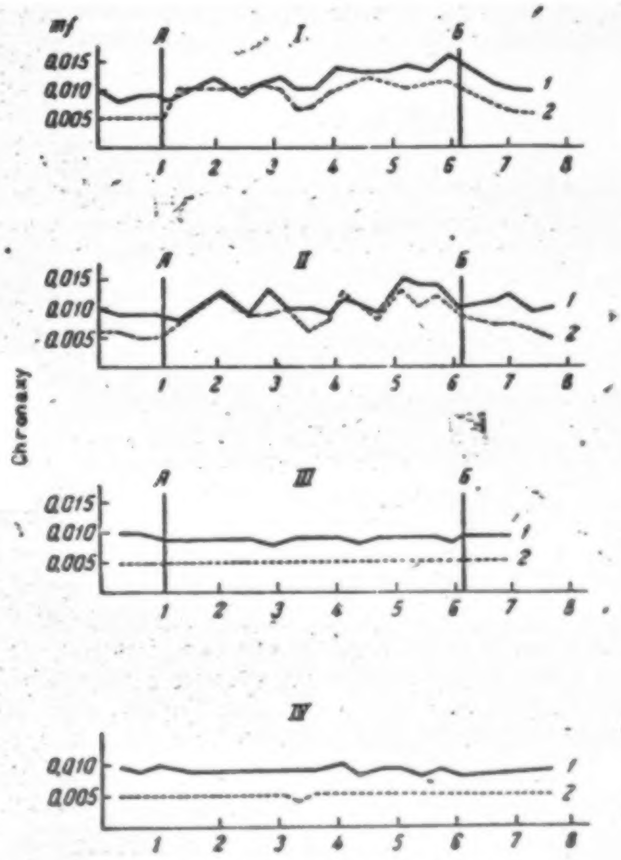

Fig. 2. Muscle antagonists chronaxy in rats during the inhalation of air containing hydrogen fluoride in animals of Groups I, II, III, and IV.
1 - extensors; 2 - flexors
AB - Inhalation period

For rats of Groups III and IV the central nervous system control functions remained within normal limits. In rats of Groups I and II changes appeared immediately in the form of delayed response and as compensation, and inverse antagonist chronaxy ratio. Chronaxy delay was especially characteristic of the extensors (38.4 percent of cases as against 30.8 for the flexors). These results were statistically reliable.

These changes in the central nervous system activity indicated profound inhibition involving the complex system of cortical and subcortical control centers. Rats of Groups I and II also exhibited statistically reliable depressed cholinesterase activity, an enzyme which transmits nerve stimulation.

The neurohistological examination of the brain, conducted by O.K. Shturkina of the Sverdlovsk Medical Institute, centered on the motor and sensory analyzers, mainly the snapses and the state of the nerve cells. Rats of Group I showed hyperemia of the membrane capillaries and of the brain matter, perivascular edema, thickening of the dendrites with formation of moniliform distensions, and disappearance of protoplasmic spines. The nerve cells suffered structural changes: uneven coloration of the Nissel substance, partial lysis thereof, and thickening, swelling, and shriveling of the protoplasm. In some nerve cells the nuclei had disappeared and neuronophagia appeared. During a 3-month recovery several changes of a compensatory nature were observed: destructive changes in the nerve cells were less pronounced, and the spines on the dendrites became less clear. This process was not fully compensated, because profound destructive changes were found in individual nerve cells with dead nuclei. It is understandable why recovery was incomplete in rats of Group I; the damage to their nervous activity was more than functional in its effect.

De Eds (1936), Roholm (1937), Thomas, Wilson, De Eds (1935) are of the opinion that changes in bone tissue, characteristic for fluorosis, are the result of impaired calcium phosphate metabolism brought about by fluoride inhibition of the enzyme phosphates. They suggested that this enzyme participated in the processes of bone formation by liberating inorganic phosphorus from phosphoric acid esters; the former, by joining a bond with calcium ions, forms calcium phosphate, which is essential for building bone tissues. Roholm (1937) attached basic importance to alkaline phosphatase in these processes. However, the literature on the effects of F on phosphatase activity presented contradictory data. The present investigation of alkaline phosphatase was conducted with 6 rats from each group. Its activity was determined by the method of G. K. Shlygin and S. Ya. Mikhlin. After an initial rise in enzyme activity among rats of Groups I and II the level dropped; the Group III level did not differ from that of the controls.

Despite fluctuations in alkaline phosphatase activity at times during inhalation, the inorganic phosphorus concentration in the blood was constant and did not exceed normal limits. This is probably related to the fact that phosphatase activity is not the only factor which supported the phosphorus level. Even in a healthy organism this activity was not at a constant intensity level, while inorganic-phosphorus concentration varied within narrow limits. Probably the mechanism responsible for this latter circumstance is complex and diverse. It has been established that phosphates migrated directly from the bone source to the blood immediately as the inorganic phosphorus concentration of the latter dropped to a lower level.

References in the literature indicated that osseus-phosphatase activity in rats decreased under the effects of F; therefore, its activity in the experimental animals was checked after experimental inhalation was discontinued. The method of A. Bogdanskiy, with slight modifications by Ye. P. Yeremin and Z. A. Kasparaskaya (195() was used to determine this activity in the tibia of 6-11 rats from each Group. The 3 HF concentrations produced no changes.

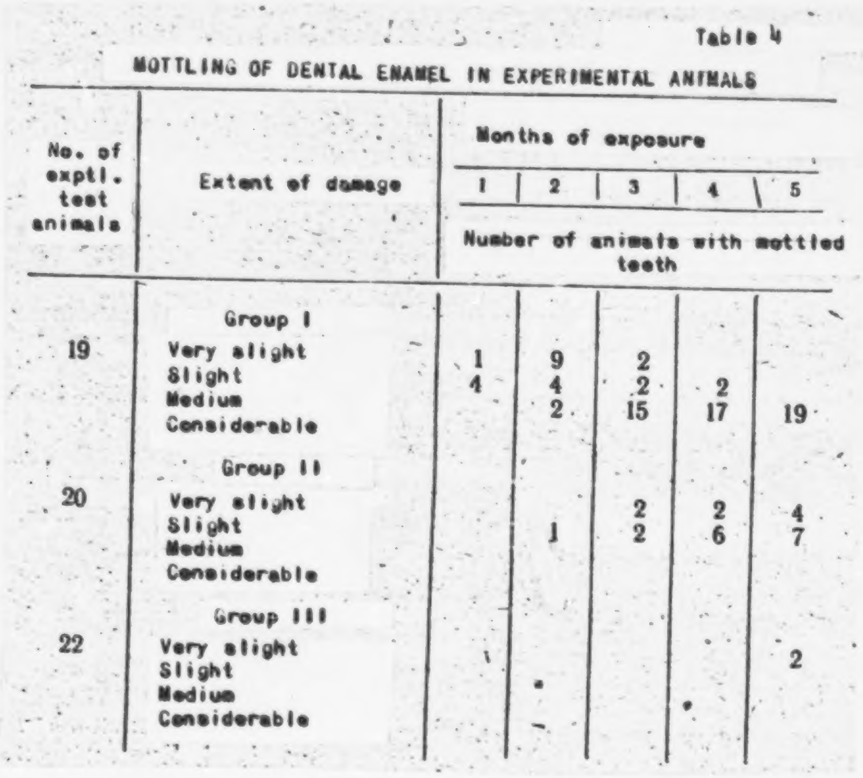

Table 4

MOTTLING OF DENTAL ENAMEL IN EXPERIMENTAL ANIMALS

No. of exptl. test animals	Extent of damage	Months of exposure				
		1	2	3	4	5
		Number of animals with mottled teeth				
	Group I					
19	Very slight	1	9	2		
	Slight	4	4	2	2	
	Medium		2	15	17	19
	Considerable					
	Group II					
20	Very slight			2	2	4
	Slight		1	2	6	7
	Medium					
	Considerable					
	Group III					
22	Very slight					2
	Slight					
	Medium					
	Considerable					

From the time that the clinical symptoms of fluorosis became known, it was necessary to assume that one of its early signs was mottling of dental enamel. A four-point system (Table 4) to evaluate tooth damage was used in this study. Observations confirmed that mottling was in fact an early specific sign of F-caused morphological changes. In white rats the color of healthy teeth is yellowish, the teeth gleam and are reminescent of amber. The teeth of five of the Group I rats after only a month's experimental inhalation became transparent, whitened, and lusterless. The severity of the damage increased as experimental inhalation continued, and by the end of the 5th month all rats had experienced some damage: the upper and lower front teeth looked like marble, were coarse and streaked, and worn down. In rats of Group II these changes were less pronounced and occurred later.

At the end of the inhalation period, enamel erosion and prism damage were discovered in Groups I and II, which also exhibited certain changes in bone tissue. Rats of Group I developed stenosis of the meduallary canal of the tubular bones, while the contours of the periostitis were uneven. In the pelvic bones there was damage to Haversian system structure, osteocyte lysis, and in the periosteum region there appeared focal resorption of the bone matter with connective tissue proliferation. Bone lime had accumulated nonuniformly. Changes in rats of Group II were less severe and were localized primarily in the lumbar region of the backbone and in the bones of the pelvis. Also noted were periostal unevenness, focal disorganization of the Haversian system, and nonuniform lime deposits. The chantes found in teeth and bone tissue are incontrovertible evidence of impairment of calcium phosphate metabolism as a result of HF inhalation.

G. Khristiani ascribed considerable significance to the level of F deposits in the skeleton and suggested that increased F content in the bones is a symptom and diagnostic factor in the early recognition of F-intoxication. He also assumed that the severity of bone changes depended on the degree of F deposition. The present author examined 6 rats from each group after inhalation was discontinued to determine F deposition in the teeth and in the femur. The teeth and bones were carefully freed of their soft tissue, dried to a constant weight, and pulverized. 0.03 g of the powder material was calcined and dissolved in 3 drops of hydrochloric acid solution, passed through an ion-exchange column with anionite AV-17. The amount of F elicited from the column was determined by the alizarintolyl method. Examination showed that HF concentrations of 0.10 and 0.03 mg/m^3 caused F to accumulate in the organism, thus confirming results of Christiani's experiment. (See Table 5, page 128).

F content in the urine was determined by an ion-exchange chromatographic method first used for such investigations in the United States (Harold, Nielsen, Logan, Utan, 1960; Talvitie, Brewer). All groups exhibited a substantially higher level of F in the urine than did the controls beginning with the 2nd month and lasting to the end of the experimental inhalation period. It is of interest to note that in spite of its high excretion level, F also accumulated in the bones.

Histological examination of the internal organs of the Group I rats after termination of the experimental inhalation, the upper respiratory paths showed severe epithilial desquamation, vascular hyperemis, and focal inflammatory lymphocytic, leucocytic, and histiocytic infiltration. Varying degrees of severe focal inflammation of the mucous membrane of the nose appeared in all animals, as well as bronchitis and peribronchitis. There was hyperemia of the pulmonary and 2 cases of 5 focal pneumonia was noted.

Table 5

FLUORIDE CONTENT IN DENTAL AND BONE TISSUES OF EXPERIMENTAL ANIMALS

Animal group	Teeth		Thighbone	
	M±m	t	M±m	t
I	110,5±5,60	17,27 (c)*	121,3±8,70	9,57 (c)
II	18,2±1,20	3,87 (b)	51,4±3,20	5,12 (c)
III	15,2±2,14	1,27 (o)	28,9±2,85	1,14 (o)
Control	12,2±0,98		23,3±4,0	

*Statistical reliability: b – 99%; c – 99.9%; 0 – Not reliable

Protein and aliphatic dystrophy were discovered in the liver; in the kidneys, protein dystrophy and increased permeability of the arterioles were seen. Plethora was observed in the vessels of the red spleen pulp, while reduction in some lymphoidal follicles and hyperplasia of the reticular cells was found in the white pulp. The walls of the central artery of the spleen had thickened as a result of plasmatic impregnation. Myocardial change was expressed as disappearance of transverse striation and disjunction of the myofibrilles.

The changes in rats of Group II were less pronounced. The nasal mucous membrane was thinner in places, the cell structure could not be clearly delineated, while in some cases, epithelial proliferation was observed. Hypersecretion and desquamation of the epithelium were observed in the bronchi. Plethora was found in the lungs and parenchymatous organs.

No histological changes were found in rats of Group III.

CONCLUSIONS

1. The odor perception threshold and the threshold of reflex action of HF in the human organism (the latter established by adaptation to the dark) are both 0.03 mg/m^3.

2. Five-month experimental inhalation HF in concentrations of 0.10 and 0.03 mg/m^3 produced many changes in warm-blooded animals:

   a. inhibition in the central nervous system, the higher concentration leading to irreversible destructive changes in the nerve cells;

   b. a change in calcium phosphate metabolism;

   c. F accumulation in the bones; and

   d. histomorphological changes in teeth, bones, and internal organs.

3. The extent of the changes varied with the HF concentration in the inhaled air.

4. An HF concentration of 0.01 mg/m^3 produced no changes in the experimental animals.

5. The investigation had shown that:

a. an HF concentration of 0.15 mg/m^3 cannot be regarded as threshold of odor perceptive concentrations;

b. An HF concentration of 0.03 mg/m^3 cannot be taken for the maximum allowable single-exposure concentration;

c. the maximum single-exposure concentration of HF must not exceed 0.02 mg/m^3.

EXPERIMENTAL BASIS FOR THE DETERMINATION OF MAXIMUM ALLOWABLE CONCENTRATION OF VANADIUM PENTOXIDE IN ATMOSPHERIC AIR

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Air pollution by vanadium pentoxide aerosol (VPO) is caused by a variety of technological processes used in several branches of the national economy. The most significant of these is the metallurgical industry, where ferro-vanadium and VPO are used as additives in producing alloy steels. Because VPO has a comparatively low melting point (675° C), its vapors escape into the air and in the form of an aerosol the particles of which run in size of up to 2 microns (I. V. Roshchin, 1952, 1963). Therefore, vanadium pentoxide pollution can affect not only industrical premises but also the atmosphere, along with other stack emissions. Vanadium pentoxide aerosol can pollute the air as a result of stack discharges omitted by boiler plants and other systems operating on coal. The lower-grade coals have a high ash content. According to V. A. Nazarenko (1937), the VPO content of ash in coal and lignite ranges between 0.44×10^{-4} and 9.3×10^{-2} percent. In petroleum fly ash, particularly petroleum with a high sulfur content, VPO content ranges between 5 to 17 percent (I. V. Roshchin, 1962; it can also be found in some of the heavy refining products, such as lubricant ash, where its content may run from 0.5 to 40 percent (Yu. P. Pal'tsev, 1964). Thus, oil-fueled heating installations and diesel motors can pollute the air through their stack emissions and tailpipe discharges (Morette, 1959).

The metal industry development, the construction of powerful thermo-electric stations, the growth of diesel-powered bus fleets in the cities, etc., are probable sources of atmospheric pollution by VPO.

Many investigators called attention to this fact in the past few years. Cholak, et al. (1952) found up to 0.2 micrograms of vanadium per cubic meter of air in Cincinnati and its environs. Morette (1959) found 11 to 1922 micrograms per gram of street dust in Paris. Dohan (1961) discovered in the air of five American cities vanadium concentrations of 0.2 to 1.2 mg/m^3. The present author conducted studies in 1963-1964 in the proximity of some large metallurgical plants and found vanadium in the atmospheric air in 87 percent of the samples; vanadium pentoxide concentrations were as follows:

Distance from Source of Emission, meters	Micrograms of Vanadium Per Cubic Meter of Air
150	1.49±0.25
500	0.47±0.09
1000	0.35±0.39
1500	0.98±0.13

In eleven percent of the positive samples VPO concentrations rose to 2 mg/m^3. According to data found in the literature, vanadium (and particularly its pentoxide) is not indifferent to the organism, causing irritation of the respiratory paths, acute and chronic inflammation of the upper respiratory tract, and lung changes (I. V. Roshchin, 1962; Ye. I. Matantseva, 1960; Kh. G. Gul'ko, 1951; Dutton, 1911; et al.). In addition, vanadium and its compounds have negatively affected the nervous system (Ye. A. Mel'nikova, 1957; K. P. Selyankina, 1961; I. V. Roshchin, 1963; Dutton, 1911; et al.), the blood (Kh. G. Gul'ko, 1951; Symanski, 1939; et al.), hemodynamics (I. V. Roshchin, 1952), and metabolism (T. A. Prokopenko, 1958; Lewis, 1959; et al.). Many authors have also noted changes in some internal organs (Ya. B. Reznik, 1954; Molfino, 1939; et al.). According to Franke and Moxson (1936, 1937) chronic exposure to vanadium compounds elicited more toxic symptoms in the organism than exposure to arsenic.

In addition, vanadium compounds in atmospheric air can act as catalyzers of sulfur dioxide present in the air and convert it to sulfuric acid aerosol (Tomas, 1959), which, according to K. A. Bushtuyeva (1961), possessed more irritating properties than the initial sulfurous anhydride. All this points to the fact that vanadium and its compounds are important components of air pollution and should be taken into account by the municipal departments of hygiene.

Among the various compounds, the most important is pentoxide, which is one of the most toxic vanadium compounds (I. V. Roshchin; 1952, 1963) it is volatile, can enter the atmosphere in relatively large quantities and at greater frequency than others. Therefore, at the recommendation of the Committee on Sanitary Protection of the Air, a study was initiated to establish a hygienic basis for the determination of the maximum average-daily concentration of VPO in atmospheric air. In this connection a study was conducted in the summer of 1964 in which 33 rats weighing 110 - 130 g were exposed to the inhalation of vanadium pentoxide continuously for 70 days. The VPO concentration for rats of Group I was 0.027±0.002 mg/m^3, which is 3.7 times below the maximum concentration permitted in industrial premises (0.1 mg/m^3). In rats of Group II it was 0.002±0.00013 mg/m^3, i.e., close to the concentrations found in the air around metallurgical plants that were not using vanadium for special purposes. Rats of Group III served as controls. Air was run into the three exposure chambers of 100 liters capacity at the rate of 30-30 li/min, assuring adequate air ventilation (V. A. Popov, 1964).

Vanadium pentoxide is most frequently encountered in air in the form of a condensate. Therefore, it was melted in a tubular furnace to generate the aerosol. The furnace temperature was stabilized at 800° C with a specially designed thermostat to assure a constant rate of evaporation. The finely dispersed aerosol was fed into the chambers by pumping air into the furnace at a rate of 2.5-3 li/min. This set-up was kept apart from the animal exposure chambers to prevent its noise from disturbing the experimental rats.

Eighteen air samples were collected from the chambers in the course of the inhalation period by using AFA-KhA filters which were decomposed in a mixture of concentrated nitric and sulfuric acids. An FEK-M photoelectric colorimeter was used for the determination of VPO according to the method of M. V. Nifontova (1960). The experimental results were processed statistically. The rats' condition before, during, and after experimental inhalation was recorded on the basis of their general condition, body weight, motor chronaxy of the antagonist muscles, blood cholinesterase activity, total protein, blood serum protein fractions, oxyhemoglobin in venous blood, and luminescence of blood leucocytes. At the end of the inhalation period rate of oxygen absorption in granulated liver tissue was determined by a Warburg device; a pathohistological examination of some internal organs was also made. During the inhalation period rats of all three groups gained weight and were active.

Motor chronaxy of muscle antagonists was included because V. A. Ryazanov (1961) has shown it to be a sensitive test in determining maximum allowable concentrations of toxic substances. An ISE-OL electrol pulse stimulator was applied every 10 days, under identical conditions, to the right hind shin. Data in Table 1 show that in rats of Group II chronaxy remained unaffected, but in rats of Group I substantial changes occurred during inhalation, such as a statistically reliable drop in extensor chronaxy (0.8 microsec on the average) and a more noticeable increase in flexor chronaxy (4 microsec). Even more striking was the change in chronaxy ratio of the antagonists in rats of Group I. As data in the Table show, an inhibitory process developed in the central nervous system on the 20th day, which as a result of the altered control function of the central nervous system on the periphery (Yu. M. Uflyand, 1941) appeared in the form of flexor and extensor chronaxy equalization. This process had become aggravated on the 30th day developing an inverse ratio which persisted to the end of the inhalation period. After 20 days of restoration the motor chronaxies, and the ratio between them, returned to their normal levels.

Vanadium pentoxide effect on the nervous system, known from the literature and confirmed by the chronaxy tests reported above, was the basis for determining cholinesterase activity, the regulation of which is largely a function of the nervous system, particularly of the cerebral cortex (D. Ye. Al'pern, 1958, 1963). The assumption here is that if VPO affects the central nervous system, the change might show up in the blood cholinesterase activity.

The method of Hestrin (1951) was used to determine gammas of acetylcholin in cholinesterase decomposition per minute. The results are summarized in Table 2.

Only rats of Group I manifested statistically reliable decrease in cholinesterase activity, which lasted through the restoration period. The other two groups exhibited no statistically reliable changes during any of the 10-day periods. Total protein and protein-fractions in the blood serum were also determined.

Administering vanadium to the animal organism caused a drop in the rate of protein synthesis (Snyder, Cornatzer, 1958) and an increase in serum gamma-globulin (Joya, 1958). We used the micromethod of Kjel'dhal to determine total blood serum protein in terms of gram-percent. Results are presented in Table 3.

Table I

MUSCLE ANTAGONISTS MOTOR CHRONAXY IN RATS DURING VANADIUM PENTOXIDE INHALATION PERIOD

	Animal group	Statistical indexes	Ten day periods of investigation					Ten day period of investigation					
			Before inhalation		During inhalation period			During inhalation period				Recovery period	
			1	2	1	2	3	4	5	6	7	1	2
Chronaxy of flexors in sec.	I	M±m	31,0± 1,73 (o)	34,7± 5,24 (o)	35,3± 7,80 (o)	45,7± 3,29 (b)	74,3± 1,34 (c)	78,3± 7,69 (b)	74,7± 9,94 (a)	94,0± 2,51 (c)	88,3± 5,37 (c)	38,5± 0,50 (a)	32,5± 1,50 (o)
	II	M±m	24,7± 3,51 (o)	26,7± 4,25 (o)	28,3± 4,91 (o)	34,7± 7,84 (o)	30,3± 4,06 (c)	26,7± 3,18 (o)	24,3± 3,67 (o)	23,7± 3,18 (o)	24,0± 3,61 (o)	22,7± 3,51 (o)	20,5± 1,50 (o)
	III	M±m	28,0± 2,31	32,0± 4,00	23,3± 2,73	30,0± 0,58	32,3± 3,67	33,3±0,89	31,0±1,00	30,3±0,34	29,3± 1,76	32,5± 0,50	29,5± 1,50
Chronaxy of extensors Time in µ sec.	I	M±m	50,0± 3,21 (o)	55,0± 7,55 (o)	39,7± 7,06 (o)	45,0± 3,79 (o)	44,3± 5,55 (o)	41,0± 5,13 (o)	27,7± 3,85 (a)	33,7± 6,33 (o)	34,7± 6,20 (o)	34,0± 7,00 (o)	51,7± 3,51 (o)
	II	M±m	41,7± 4,25 (o)	49,3± 5,46 (o)	45,0± 7,77 (o)	58,0± 11,1 (o)	48,0± 6,03 (o)	43,7± 5,24 (o)	41,7± 8,11 (o)	41,0± 3,21 (o)	41,3± 3,33 (o)	40,0± 8,00 (o)	35,0± 4,00
	III	M±m	43,0± 1,73	45,7± 3,93	38,0± 4,51	45,3± 1,67	49,0± 3,21	51,3±0,89	49,0±3,79	48,7±1,76	46,0± 2,80	48,7± 4,51	44,3± 1,53
Ratio indexes of flexors to extensors (X 100)	I	M±m	63± 0,89 (o)	62± 1,73 (o)	86± 5,00 (b)	108± 9,40 (a)	172± 19,7 (b)	193±14,5 (c)	270± 30,0 (b)	293± 40,0 (b)	263± 31,8 (b)	118± 72,0 (o)	63± 1,00 (o)
	II	M±m	61± 2,41 (o)	60± 5,13 (o)	64± 6,90 (o)	60± 3,48 (o)	63± 4,06 (o)	63± 5,70 (o)	60± 4,40 (o)	58± 4,20 (o)	59± 4,40 (o)	57± 3,00 (o)	61± 10,0 (o)
	III	M±m	65± 2,31	70± 3,53	60±1,86	662±2,70	66± 4,10	65±0,55	64±3,18	63±4,50	64±1,53	68±5,50	66±1,00

Notes: Statistical reliability: a - 95%; b - 99%; c - 99.9%; 0 - Not reliable

Table 2

BLOOD CHOLINESTERASE ACTIVITY IN RATS IN GAMMAS OF ACETYLCHOLIN HYDROLYZED PER 1 ML OF BLOOD PER MINUTE)

Period	Statistical indexes	Animal groups		
		I	II	III Control Group
Before inhalation	M ± m	120,0±7,20 (o)*	121,7±7,45 (o)	127,7±3,13
During inhalation	M ± m	98,1±4,95 (a)	119,4±3,33 (o)	119,1±5,53
Recovery period	M ± m	84,0±4,62 (a)	104,8±3,95 (o)	100,0±6,77

*Statistical reliability: a - 95%; 0 - Not reliable

Table 3

TOTAL BLOOD PROTEIN CHANGES IN WHITE RATS DURING PERIOD OF VANADIUM PENTOXIDE INHALATION

Period	Statistical indexes	Animal groups		
		I	II	III Control Group
Before inhalation	M ± m	6,00±0,15 (o)*	4,80±0,73 (o)	5,75±0,00
During inhalation	M ± m	6,17±0,03 (b)	6,67±0,53 (o)	6,65±0,05

*Statistical reliability: b - 99%; 0 - Not reliable

It was established that after 40 days of inhalation the serum protein in rats of Group I was virtually at the same level as before the experimental inhalation, while in rats of Groups II and III (controls) there was an increase incident to the normal growth of the organism. S. D. Balakhovskiy and I. S. Balakhovskiy (1953) are of the opinion that total blood protein in young rats constituted 4.8% of their body weight and 7.2% in mature rats. In other words, VPO inhalation inhibited protein synthesis in rats of Group I, the difference from the controls being statistically reliable.

Blood serum protein fractions were determined by the method of paper electrophoresis. Results are summarized in Table 4.

The albumin/globulin index remained relatively constant. Protein fractions did not differ significantly from those of the controls, whereas in rats of Group I there was a statistically reliable increase in globulin by the 40th day of inhalation, accompanied by a reliable globulin decrease.

The method of luminescent microscopy of leucocytes with vital staining of the specimens had been used in establishing maximum tolerable toxic concentrations of air pollutants (M. I. Gusev, K. N. Chelikanov, 1963).

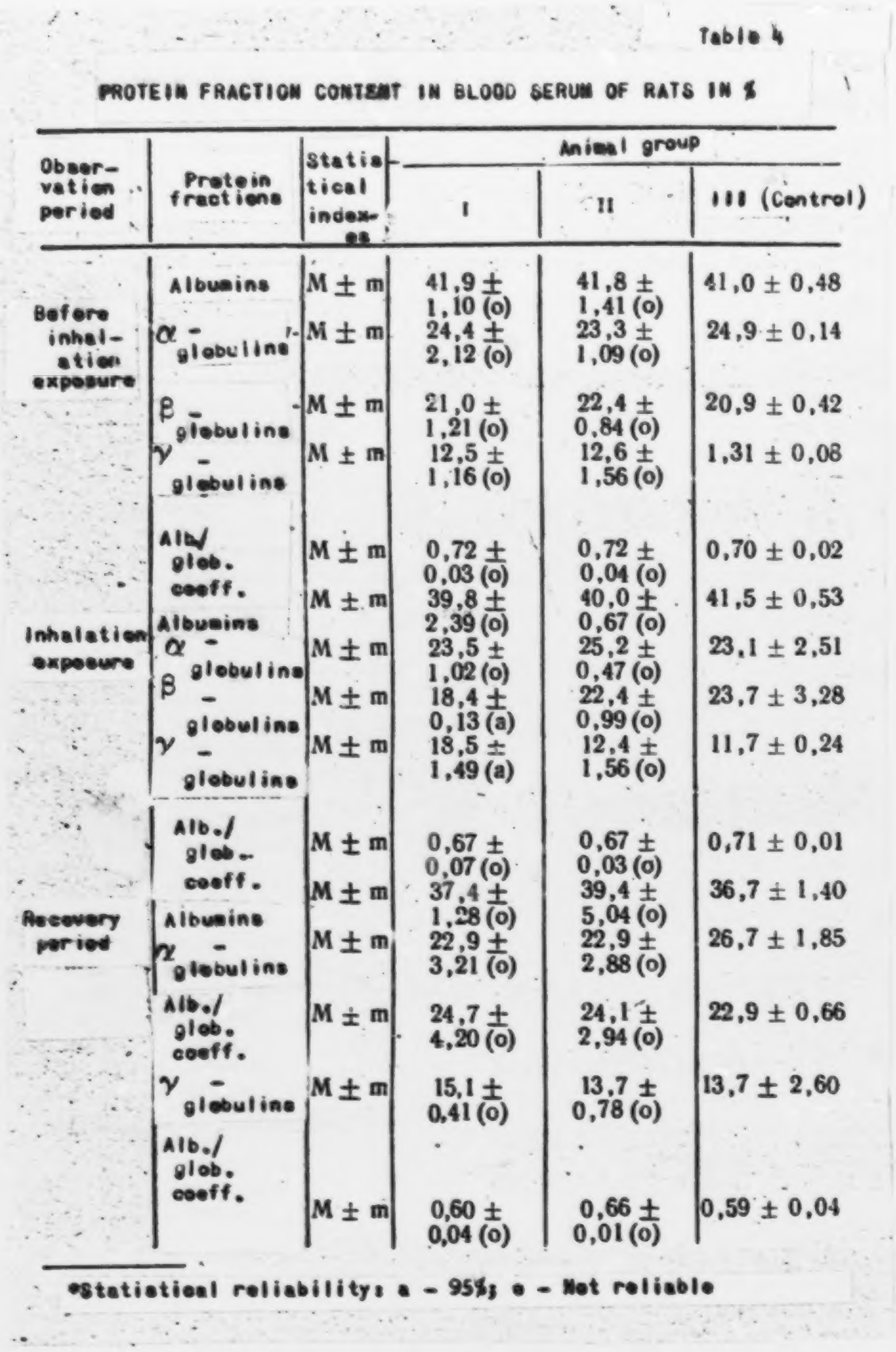

Table 4

PROTEIN FRACTION CONTENT IN BLOOD SERUM OF RATS IN %

Observation period	Protein fractions	Statistical indexes	Animal group		
			I	II	III (Control)
Before inhalation exposure	Albumins	M ± m	41,9 ± 1,10 (o)	41,8 ± 1,41 (o)	41,0 ± 0,48
	α - globulins	M ± m	24,4 ± 2,12 (o)	23,3 ± 1,09 (o)	24,9 ± 0,14
	β - globulins	M ± m	21,0 ± 1,21 (o)	22,4 ± 0,84 (o)	20,9 ± 0,42
	γ - globulins	M ± m	12,5 ± 1,16 (o)	12,6 ± 1,56 (o)	1,31 ± 0,08
	Alb/ glob. coeff.	M ± m	0,72 ± 0,03 (o)	0,72 ± 0,04 (o)	0,70 ± 0,02
Inhalation exposure	Albumins	M ± m	39,8 ± 2,39 (o)	40,0 ± 0,67 (o)	41,5 ± 0,53
	α - globulins	M ± m	23,5 ± 1,02 (o)	25,2 ± 0,47 (o)	23,1 ± 2,51
	β - globulins	M ± m	18,4 ± 0,13 (a)	22,4 ± 0,99 (o)	23,7 ± 3,28
	γ - globulins	M ± m	18,5 ± 1,49 (a)	12,4 ± 1,56 (o)	11,7 ± 0,24
	Alb./ glob. coeff.	M ± m	0,67 ± 0,07 (o)	0,67 ± 0,03 (o)	0,71 ± 0,01
Recovery period	Albumins	M ± m	37,4 ± 1,28 (o)	39,4 ± 5,04 (o)	36,7 ± 1,40
	α - globulins	M ± m	22,9 ± 3,21 (o)	22,9 ± 2,88 (o)	26,7 ± 1,85
	Alb./ glob. coeff.	M ± m	24,7 ± 4,20 (o)	24,1 ± 2,94 (o)	22,9 ± 0,66
	γ - globulins	M ± m	15,1 ± 0,41 (o)	13,7 ± 0,78 (o)	13,7 ± 2,60
	Alb./ glob. coeff.	M ± m	0,60 ± 0,04 (o)	0,66 ± 0,01 (o)	0,59 ± 0,04

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*Statistical reliability: a - 95%; o - Not reliable

The present author employed the supravital staining with acridine orange in 1:20,000 dilution (Ye. B. Zakrzhevskiy and L. G. Vasil'yeva, 1963). Specimens prepared in this way did not lose the advantages of vital staining and at the same time prolonged the time during which the staining method could be applied. Normal and changed leucocytes were differentiated by the luminescence intensity of their nuclei. This method depends on the extent of fluorochrome molecule polymerization (Ye. B. Zakrzhevskiy and L. G. Vasil'yeva, 1963), which in turn depends on the condition of the DNA and RNA molecules in the protoplasm and in the cell nucleus. Of considerable importance is the degree of polymerization of the nucleic acids themselves, the nature of the complex bond with acridine orange, and the chemical structure of the nucleic acids. Nondenatured nucleic acids have a helical structure and emanate green luminescence, while denatured ones have the shape of a single spiral and emanate red luminescence.

In the early stages of DNA reorganization in the cell nucleus the luminescence is yellow instead of the normal green, which later changes to orange and then to red. Normal blood has only occasional leucocytes with altered luminescence, which must be related to their destruction.

Table 5

PERCENT OF LEUCOCYTES WITH CHANGED LUMINESCENCE

Observation period	Statistical indexes	Animal group		
		I	II	III Control
Before inhalation	M±m	1,25±0,70 (o)	1,25±0,70 (o)	1,25±0,70
Inhalation				
After 10 - 20 days	M±m	5,75±0,75 (b)	2,00±0,82 (o)	2,25±0,25
45 days	M±m	13,75±3,84 (a)	1,75±0,75 (o)	1,25±0,25
70 days	M±m	20,25±5,40 (a)	1,25±0,25 (o)	1,25±0,25
Recovery period	M±m	1,25±0,63 (o)	1,00±0,58 (o)	1,50±1,50

Note: Statistical reliability: a - 95%; b - 99%; c - 99.9% ; 0 - Not reliable

Results of this study are summarized in Table 5, and indicate that there were no substantial changes in rats of Groups II and III at any time. In rats of Group I the number of leucocytes with yellow nucleic luminescence increased, and cells with orange and red nuclei appeared. This change became statistically reliable on the 20th day of the inhalation test and continued to increase during the experiment reaching the normal level only after the 20th recovery day.

Some authors are of the opinion that vanadium in the organism can affect the redox processes (Trummert, Bochm, 1957; Snyder, Cornatzer, 1958; Mitchell, 1953. Hyperventilation of the lungs occurs when VPO concentrations in industrial premises exceeded the maximum permissible concentration (Ye. I. Matantseva, 1960), and in an experiment with animals (K. P. Selyankina, 1961) vanadium was found to increase oxygen demand as recorded by the Mitropol'skiy apparatus. In this connection it was thought apropos to employ oxyhemometrics in the present experiment. Blood was taken from the caudal vein. A model 0-57 oxyhemometer was used to measure oxyhemoglobin content in percent. Results are presented in Table 6.

Table 6

PERCENT OF HEMOGLOBIN IN RATS' VENOUS BLOOD

Observation day	Statistical indexes	Animal group		
		I	II	III Control
Before inhalation	M±m	60,0±1,8 (o)*	60,0±1,8 (o)	60,0±1,8
Inhalation period	M±m	51,5±2,9 (b)	59,6±2,1 (o)	61,2±1,3
Recovery period	M±m	63,0±1,0 (o)	62,0±2,0 (o)	59,0±2,0

*Statistical reliability: b - 99%; 0 - not reliable

Because of a certain disunity character of the data it was necesary to obtain the average oxyhemoglobin content in the blood of rats in all three groups. Statistically reliable changes were found only in rats of Group I; no such changes were observed in rats of Groups II and III. The oxyhemoglobin content in rats of Group I returned to normal after the 20th day of the recovery period.

An experiment conducted by F. and M. Berngeym (A. O. Boykar, 1953) also sheds some light on the role of vanadium in the redox processes in the organism. The authors showed that introduction of metavanadate and vanadium acetate in amounts up to and including 10-20 g of vanadium enhanced tissue respiration by the granulated liver in higher animals, raised oxygen demand by a factor of 10. At the end of the inhalation period tests were conducted to determine the extent of VPO's effect on tissue respiration. Eleven rats from the three groups were decapitated and their livers were removed. Respiration of the granulated liver tissue was studied in a Warburg device. The results are shown in Table 7.

Table 7

OXYGEN CONSUMED BY 500 MG OF GROUND LIVER IN WARBURG APPARATUS IN 20 MINUTES (IN MICROLITRES)

Statistical Indicater	Animal group		
	I	II	III Control
M±m	263,3±14,9 (a) 124%	222,0±11,9(o) 104%	212,3±4,26 100%

Note: Statistical reliability: b - 95%; o - not reliable

Oxygen demand in rats of Group I was 24 percent higher than in the control rats, a substantially lower increase than the one reported by the Berngeyms, in whose experiment vanadium compounds were added directly to the granulated tissue in vitro, whereas in the present study the rats inhaled the pentoxide. Oxygen demand in rats of Group II did not differ reliably from the controls.

Histological examination of certain internal organs found pathological changes only in rats of Group I. The lungs showed the presence of acute stagnant vascular plethora, focal hemorrhage, and signs of panbronchitis; in the liver were found acute plethora of the central veins, surrounded by isolated small hemorrhages around them, individual infiltrates between the hepatic lobules, and signs of granular, cellular dystrophy. The kidneys exhibited pronounced granular dystrophy of the epithelium of the contorted tubicles, which in some sectors showed signs of necrosis. There was acute myocardial vascular plethora surrounded by focal hemorrhages.

Results of all tests show that a vanadium pentoxide concentration of 0.027 mg/m^3 in the air produced in the chronic experiment functional and morphological changes in the organism of the test animals; a concentration of 0.002 mg/m^3 had no such effect.

It should be noted that the two VPO concentrations differed by the disparity between the two test concentrations (almost a factor of 10). This author conducted in 1964 a second series of tests with an intermediate concentration close to the inactive level, using 20 young white male rats divided into a control group and a group subjected to continuous 40-day inhalations of air containing 0.006±0.0056 mg/m^3 of VPO.

Two tests were conducted in the first series to determine functional changes: muscle antagonist chronaxy and leucocyte luminescence, and records of body weight and general well being. Four rats from each group were used in the motor chronaxy test. During the first month of inhalation there were no statistically reliable changes in the chronaxies but at the end of that month some small but significant shifts were found in the chronaxy ratio of extensors and flexors.

Table 8

PERCENT OF LEUCOCYTES WITH CHANGED LUMINESCENCE OF NUCLEI (SECOND SERIES)

Observation period	Statistical Indicators	Animal group	
		Experimental	Control
Before inhalation	M±m	0,67±0,33 (o)	0,67±0,33
Inhalation			
After 15 days	M±m	1,50±0,56 (o)	0,60±0.40
After 30 days	M±m	3,17±1,25 (o)	1,67±0,42
After 40 days			
After starvation	M±m	4,83±0,79 (b)	1,00±0.37

Note: Statistical reliability: b - 99%; o - not reliable

Leucocyte luminescence was studied in 6 rats from each group (Table 8). During the first month there were no significant changes.

Initial shifts appear in the animal organism more clearly under functional loading. With this in mind the rats were subjected to a 3½-day fast during the 6th week of experimental inhalation giving them only water. Tests made at the end of the fast revealed substantial changes in the functional condition of the test animals. There was a statistically reliable change in the chronaxy ratio of shin flexors and extensors, and the same was true of leucocyte luminescence, a 5-fold shift occurring by comparison with the controls.

CONCLUSIONS

1. Chronic, 24 hr., 70-day inhalation by rats of air-polluted with a VPO concentration of 0.027 mg/m^3 of vanadium pentoxide induces substantial changes in the functional state of the organism, as well as morphological changes in some organs.

2. A concentration of 0.006 mg/m^3 produced slight functional changes which with functional loading in the form of starvation became aggravated.

3. A VPO concentration of 0.002 mg/m^3 proved inactive.

4. On the basis of this investigation it is recommended that the maximum average-daily allowable concentration for VPO in atmospheric air be set at 0.002 mg/m^3.

AN HYGIENIC STANDARDIZATION OF ALPHAMETHYLSTYRENE IN ATMOSPHERIC AIR

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Modern chemical industry is characterized by the progressively increasing production of synthetic compounds and substances and by changes and improvements in their production processes and quality. In the course of the 1958-1965 seven-year period production of synthetic rubber increased by a factor 3-4. In this connection it should be noted that a modern divinylmethylstyrene synthetic-rubber plant is capable of polluting the air around it with alphamethylstyrene (AMS) vapors over a radius of 4000 m.

AMS is a homolog of benzene with one unsaturated bond in the side chain. It is a colorless or slightly yellowish fluid having a boiling point of 162-163° C and a characteristic odor of naphthalene (N. V. Lazarev, 1963). Some authors investigated its toxic effects in chronic experiments.

A. I. Korbakova (1961) and E. A. Kapkayeva (1963) discovered that exposure to AMS vapors in concentrations of 10-40 and 5-50 mg/m^3 for 5-6 hrs. daily over 6 months affected the functional state of the liver, kidneys, and central nervous system of white rats. They also found slight changes in the peripheral blood of animals exposed to 50 mg/m^3 of AMS vapors. T. A. Blinova (1953, 1954) found that different AMS inhalation parameters (100 mg/m^3, 6-7 hrs. daily for 30 days) had a myelotoxic effect in the peripheral blood of rabbits; erythropenia, leucotosis with a small shift to the left, monocytosis, and reduced reticulocyte count. Workers engaged in synthetic rubber production chronic intoxication with AMS vapors plus divinyl developed an asthenovegetative syndrome and cerebral asthenia of a nonspecific type. Hypotonia and hypothermia have been noted in workers at the end of a work week (E. A. Kapkayeva, 1963; Z. A. Yermolova et al., 1964).

The maximum average-daily allowable concentration of AMS vapors in atmospheric air was determined next. Sixty white rats weighing 140-170 g were divided into 4 groups of 15 rats each. Rats of Group I were exposed to the inhalation of air containing 5 mg/m^3 of AMS 24 hrs. daily for 90 consecutive days, which is the maximum allowable AMS concentration in indoor working premises; for rats of Group II - 0.5 mg/m^3; and for rats of Group III - 0.05 mg/m^3; Group IV served as controls. Each group was divided into three subgroups of five rats each for investigation of blood, biochemistry, and physiology. Before experimental inhalation rats were observed for 45 days of background study. The experimental inhalation period extended over three months followed by a one month's recovery period. Experimental data were processed statistically.

AMS effect on the functional state of the central nervous system was evaluated on the basis of the latent period of the unconditioned defensive reflex reaction of the time required for a stimulus to be transmitted over a reflex area.

It has been established by Soviet and foreign authors (V. V. Zakusov, 1957; S. I. Gorshkov, 1964; P. Tasaki, 1948) that the latent period of an unconditioned reflex reaction in laboratory animals was a fine and sensitive differentiation of functional changes in the central nervous system activity resulting from chemical and physical stimuli, due to the fact that central synapses and cells constituted the units in which the most pronounced quantitative changes appeared.

According to S. I. Gorshkov (1962), "The latent period of a reflex reaction reflected the functional state of an individual tissue at the moment it generated a single excitory wave, more characteristically than did chronaxy."

Precise evaluation of this reaction requires that the following conditions be observed: simultaneous initial reading of latent-period from the moment of stimulation and cessation of counter operation the moment a response is generated; stimulation must be instantaneous; it must be in effect only for the shortest imperative time. A reflexometer proposed by Gorshkov (1964) satisfied these requirements (Fig. 1).

The following method was used in this study. The rat was placed into the chamber with its extremities in contact with parallel metal plates. The chamber cover and the counter were placed on the animal's spine. After the time recorder was switched on a threshold-tension current was fed in for the production of unconditioned electrocutaneous (painful) stimuli. At the threshold tension the rat was startled, the switch on the cover was shorted, the contact was broken, and the counter ceased to operate. The time elapsed between the moment of the stimulus (place where the mobile contact was placed) and the arrow on the counter stopped moving (determined on the scale of the device) is the (LPURDR) latent period of the unconditioned reflex defense response.

In one series of tests 5 readings were made with a single current tension at intervals of 2-3 min. (to avoid trade effects). The small differences in the readings were regular and constituted a functional characteristic of the central nervous system. The arithmetic mean of all readings is the LPURDR.

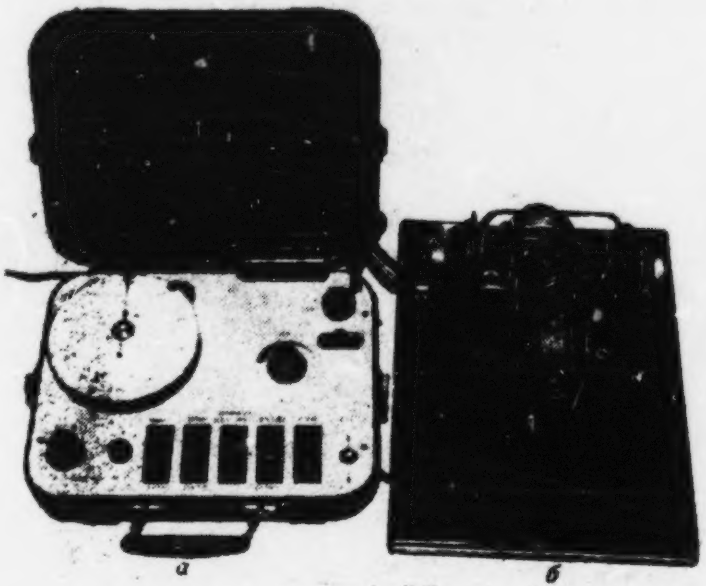

Fig. I. Reflexotonometer
a - Computer; b - Animal chamber

The AMS-LPURDR tests were made weekly on 5 rats from each group. During the 5th week rats of Group I (5 mg/m^3) manifested statistically reliable depression of the functional state of the central nervous system, expressed as an increase in LPURDR (Fig. 2). By the end of the recovery period the latent time returned to normal. No changes were noted in rats of Groups II and III.

Once every two weeks blood samples were taken to determine the contents of nucleic acids, leucocytes, erythrocytes, and hemoglobin, and changes in the leucocyte luminescence. Erythrocyte counts and hemoglobin were determined by an erythrometer; leucocytes were counted in a Goryayev chamber. The concentrations used in the tests caused no changes in the three parameters employed.

References in the literature indicated that without replacing other forms of microscopy (ordinary, phase-contrast, electron, interference, UV, etc.), luminescent microscopy offered additional advantages for biological and medical investigations (Ye. B. Zakrzhevskiy, L. G. Vasil'yeva, 1963). A. D. Semenko (1963) studied changes in leucocyte coloration caused by atmospheric pollutants. M. I. Gusev and K. N. Chekalinov (1963) were the first to apply this method in establishing hygienic norms for toxic air pollutants, directing their efforts to pentane in a chronic experiment with animals.

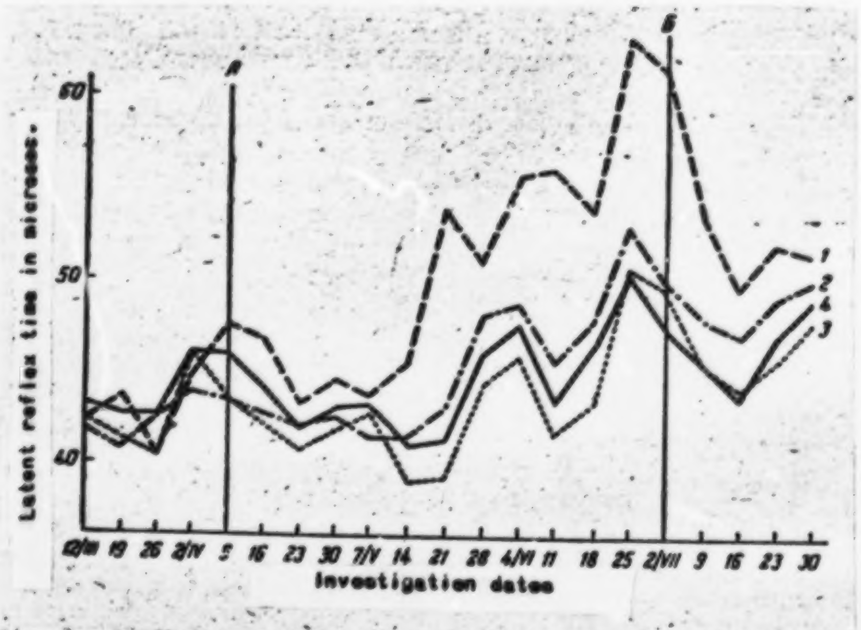

Fig. 2. Changes in latent reflex period effected by alphamethyl - styrol
1 - 5 mg/m³, group I; 2 - 0.5 mg/m³, group II; 3 - 0.05 mg/m³, group III; 4 - Control group. AB - Inhalation period.

In this connection the present investigation included fluro-chromed vital liquid specimens, acridine orange in a physiological solution in 1:100,000 dilution serving as the fluorochrome. An ON-18 luminescent illuminator was used as the source of light stimulation.

Rats of Groups I and II manifested altered leucocyte staining properties from normal green to yellow or orange-yellow, the changes in Group I being more clearly defined and faster stained. Group III did not differ from the controls (Fig. 3).

Attention of many investigators is at present being directed to the nucleic acids, the biological functions of which is closely related to protein biosynthesis, to the mechanisms of normal and pathological growth and development, to heredity, and to biological specificity.

The nucleic acids are high-molecular organic compounds found in all biological entities and cells of the organism, and are their constituent element. However, up to the present no one has applied them to studies similar to the present one. The present author employed the spectrophotometric method of total quantitative determination of the nucleic acids (RNA and DNA) proposed by A. S. Spirin (1958), in which nucleic acids are extracted with hot perchlorate and determined photometrically in the UV band at two wavelengths (270 and 290 millimicrons). The procedure described by P. V. Simonov in 1960-62 was followed in making quantitative blood analyses.

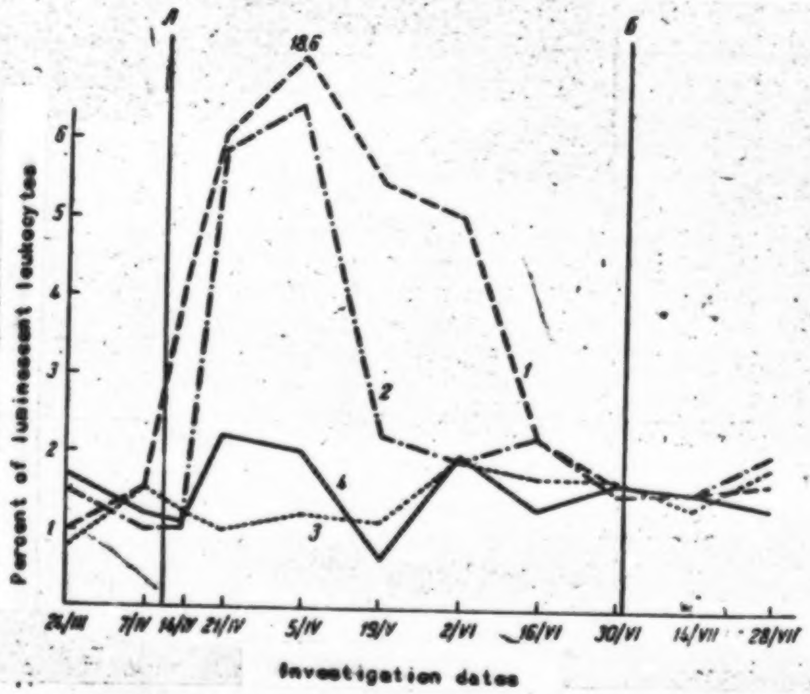

Fig. 3. Effect of alphamethylstyrol vapor on the number of blood leucocytes with changed fluorescence.
Notations same as in Fig. 2.

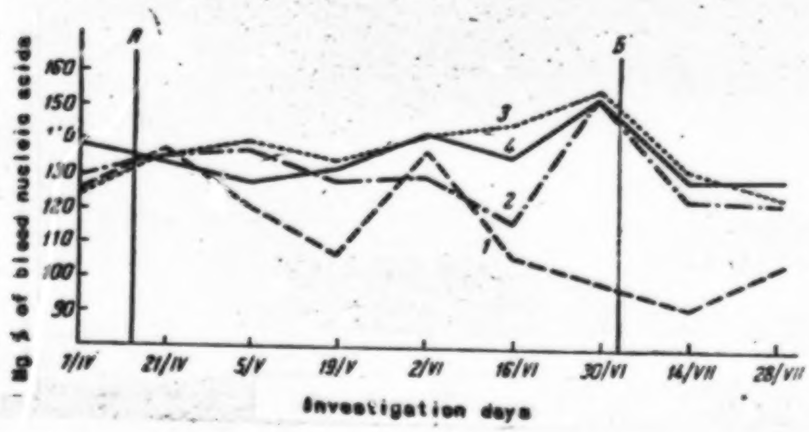

Fig. 4. Effect of alphamethylstyrol on the content of blood nucleic acids.
Notations same as in Fig. 2.

The results, graphically presented in Fig. 4, show that by the 4th week of inhalation the Group I AMS concentration caused a drop in total nucleic-acid content; after a brief remission a new reduction set in which did not return to normal during the recovery period. In Groups II and III the amount of nucleic acid in the rats' blood did not differ from the controls.

AMS effect on porphyrin metabolism was judged on the basis of changes in the rate of coproporphyrin elimination via the daily urine by 5 rats, determined once every two weeks. The amount of coproporphyrin was determined by the method described by M. I. Gusev and Yu. K. Smirnov (1960).

Decrease in rate of coproporphyrin elimination via the urine was noted on the 2d week of experimental inhalation; this decrease leveled off in the course of the inhalation experiment; it remained above the initial level even at the end of the recovery period. The change in Group II was less pronounced, and Group III did not differ from the controls. Results are summarized in Fig. 5.

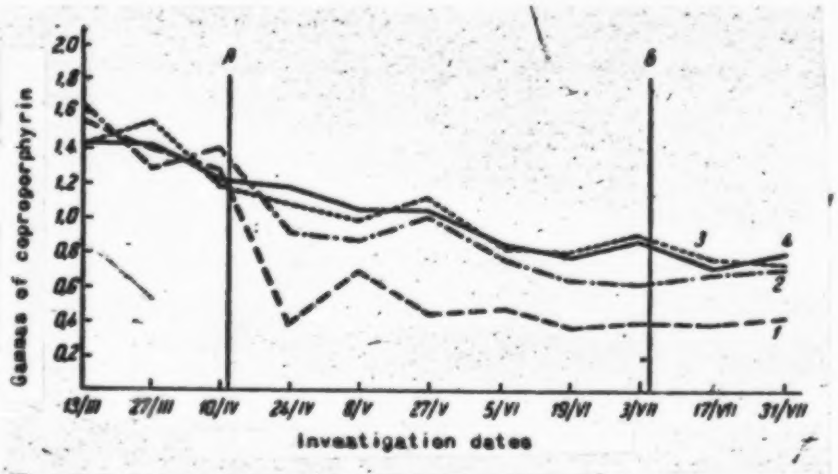

Fig. 5. Effect of alphamethylstyrol vapor on urinary coproporphyrin.
Notations same as in Fig. 2.

None of the three AMS concentrations used caused any changes in the weight and behavior of the animals during the chronic experiment.

After experimental inhalation 5 rats in each group were sacrificed. Pathomorphological and histological examinations of the organs showed that an AMS concentration of 5 mg/m³ caused changes in the lungs, kidneys, heart, and liver.

On the basis of the foregoing the present author concludes:

1. Alphamethylstyrene vapors in concentration of 5 mg/m³ and lower had no effect on the behavior, weight, and general blood picture

(leucocyte, erythrocyte, hemoglobin count) of the test animals.

2. An AMS concentration of 5 mg/m^3 depressed the central nervous system (increased reflex time), altered the biochemical reactions (reduced the content of nucleic acids in the blood and of coproporphyrin in the urine), and changed the luminescent properties of the leucocytes.

Functional changes were confirmed by pathohistological changes in lungs, liver, heart, and kidneys.

3. In concentration of 0.5 mg/m^3 AMS altered only the luminescent properties of the leucocytes, while 0.05 mg/m^3 had no effect.

4. The maximum average tolerance allowable concentration of AMS vapors in atmospheric air can be set at the level of its accepted single-exposure allowable concentration of 0.04 mg/m^3 which is the inactive level in chronic inhalation exposure of the experimental animals.

APPENDIX

MAXIMAL ALLOWABLE CONCENTRATIONS OF HARMFUL SUBSTANCES IN ATMOSPHERIC AIR OF INHABITED AREAS

Name of Air Pollutant	Maximal Allowable Concentrations	
	Maximal Single	Average 24-hours
1. Acrolein	0,30	0,10
2. Alphamethylstyrol	0,04	0,04
3. Alphanapthaquininone	0,008	—
4. Amylacetate	0,10	0,10
5. Amylene	1,5	1,5
6. Ammonia	0,2	—
7. Anilin	0,05	0,03
8. Acetone	0,35	0,35
9. Acetophenone	0,003	0,003
10. Benzene	1,5	0,80
11. Benzene, crude oil, low in S, on the basis of C	5,0	1,5
12. Benzene, shale oil, on the basis of C	0,05	0,05
13. Butyl acetate	0,1	0,1
14. Butylene	3,0	3,0
15. Vynyl acetate	0,2	0,2
16. Hexamethylenediamine	0,001	0,001
17. Hyeroperoxide of isopropyl benzene	0,007	0,007
18. Divinyl	3,0	1,0
19. Dichloroethane	3,0	1,0
20. Dimethylformamide	0,03	0,03
21. Dinyl	0,01	0,01
22. Isopropylbenzene	0,014	0,014
23. Nitric acid, HNO_3, on the basis of H	0,4 0,006	— —
24. Sulfuric acid, H_2SO_4 on the basis of H	0,3 0,006	0,1 0,002
25. Hydrochloric acid, on the basis of H	0,2 0,006	— —
26. Xylo	0,2	—
27. Metanol	1,0	0,5
28. Methylacetate	0,07	0,07
29. Malcin anhydride	0,5	0,05
30. Methylmetacrylate	0,1	0,1

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*Approved by the Chief Sanitary Officer of the USSR, 1965.

APPENDIX CONT'D.

Name of Air Pollutant	Maximal Allowable Concentrations	
	Maximal Single	Average 24-hours
31. Manganese and its compound, in tars of MnO_2	—	0,01
32. Arsenic (Inorganic compounds) (Hydrogen arsenide excluded)	—	0,003
33. Nitrobenzene	0,008	0,008
34. Carbon Monoxide	6,0	1,0
35. Oxides of nitrogen, on the basis of N_2O_3	0,3	0,1
36. Oxide of ethylene	0,3	0,03
37. Pyridine	0,08	0,08
38. Propylene	3,0	3,0
39. Dust, nontoxic	0,5	0,15
40. Vanadium pentoxide	—	0,002
41. Mercury, metallic	—	0,0003
42. Sulfur dioxide	0,5	0,15
43. Hydrogen sulfide	0,008	0,008
44. Carbon bisulfide	0,03	0,01
45. Lamp Black	0,15	0,05
46. Lead and its compounds, (tetraethylead excluded) as Pb	—	0,0007
47. Lead sulfide	—	0,0917
48. Styrol	0,003	0,003
49. Toluidineisocynate	0,05	0,02
50. Toluol	0,6	—
51. Formaldehyde	0,035	0,012
52. Phosphoric anhydride	0,15	0,05
53. Phthalic anhydride	0,2	0,2
54. Fluorine compounds, computed on the basis of F	0,03	0,01
55. Hydrofluoride	0,02	0,01
56. Phenol	0,01	0,01
57. Furfurol	0,05	0,05
58. Chlorine	0,1	0,03
59. Chlorobenzene	0,1	0,1
60. Chloroprene	0,25	0,08
61. Chromium, hexavalent, on the basis of CrO_3	0,0015	0,0015
62. Epichlorohydrin	0,2	0,2
63. Ethanol hydrolytic, on the basis of ethanol	5,0	5,0
64. Ethylacetate	0,1	0,1
65. Ethylene	3,0	3,0
66. Cyclohexanol	0,06	0,06
67. Cyclohexanon	0,04	0,01

Notes:

1. In cases of simultaneous presence in the air of more than one pollutant the MAC of which are subject to the summation principle, the sum of their concentrations must be computed by the formula discussed in Note 2. The summation must not exceed 1 in the following combinations:

a) Sulfur dioxide and sulfuric acid aerosol;
b) Hydrogen sulfide, carbon disulfide and dinyl;
c) Isopropylene benzene and hydroperoxide of isopropylene benzene;
d) Ethylene, propylene, butylene and amylene;
e) Sulfuric acid, hydrochloric acid and nitric acid (on the hydrogen ion basis);
f) Acetone and phenol;
g) Acetone and acetophenone.

2. Computation formula:

$$X = A/M_1 + B/M_2 + C/M_3 + D/M_4 + \text{etc.}$$

where X is the sought summation concentration and

$$A/M_1 + B/M_2 + C/M_3$$

are the concentrations of pollutant components divided by their corresponding MAC.

3. Where the atmospheric air contained hydrogen sulfide and carbon disulfide their corresponding integral MAC values and not their fractional values should form the computation basis.